Aerosol and Air Quality Research, 13: 350–359, 2013

ISSN: 1680-8584 print / 2071-1409 online
doi: 10.4209/aaqr.2012.05.0124

Applying the Membrane-Less Electrolyzed Water Spraying for Inactivating Bioaerosols

Chi-Yu Chuang[1], Shinhao Yang[2*], Hsiao-Chien Huang[2], Chin-Hsiang Luo[3], Wei Fang[1], Po-Chen Hung[4], Pei-Ru Chung[5]

[1] *Department of Bio-Industrial Mechatronics Engineering, National Taiwan University, Taipei 10617, Taiwan*
[2] *Center for General Education, Toko University, Chiayi 61363, Taiwan*
[3] *Department of Safety, Health and Environmental Engineering, Hungkuang University, Taichung 43302, Taiwan*
[4] *Institute of Occupational Safety and Health, Council of Labor Affairs, Taipei 10346, Taiwan*
[5] *Union Safety Environment Technology Co., Ltd, Taipei 10458, Taiwan*

ABSTRACT

The inactivating efficiency using membrane-less electrolyzed water (MLEW) spraying was evaluated against two airborne strains, *Staphylococcus aureus* and *λ virus* aerosols, in an indoor environment-simulated chamber. The air exchanged rate (ACH) of the chamber was controlled at 0.5 and 1.0 h^{-1}. MLEW with a free available chlorine (FAC) concentrations of 50 and 100 ppm were pumped and sprayed into the chamber to treat microbial pre-contaminated air. Bioaerosols were collected and cultured from air before and after MLEW treatment. The first-order constant inactivation efficiency of the initial counts of 3×10^4 colony-forming units (CFU or PFU)/m^3 for both microbial strains were observed. A higher FAC concentration of MLEW spraying resulted in greater inactivation efficiency. The inactivation coefficient under ACH 1.0 h^{-1} was 0.481 and 0.554 (min^{-1}) for *Staphylococcus aureus* of FAC 50 and 100 ppm spraying. In addition, increasing the air exchange rate also improved the inactivation rate. The inactivation coefficient of FAC 100 ppm spraying for *Staphylococcus aureus* was 0.412 and 0.403 (min^{-1}) under ACH 1.0 and 0.5 h^{-1}. These results indicated that MLEW spraying is likely to be effective in minimizing microbial airborne contamination, especially for poorly ventilated spaces.

Keywords: Membrane-less electrolyzed water; Free available chlorine; Indoor; Bioaerosols; Inactivating efficiency.

INTRODUCTION

Individuals spend an average of 87.2% of their time indoors (Lance, 1996), highlighting the increasing importance of indoor air quality. Exposure to bioaerosols such as bacteria and its related toxin in indoor environment (Lee *et al*., 2012; Singh *et al*., 2011) may result in sick building syndrome (SBS), such as lower respiratory irritation, allergic rhinitis, couch and asthma. (Eduard *et al.*, 1993; Koskinen *et al.*, 1994; Melbostad *et al.*, 1994). The airborne transmission of nosocomial bacteria not only poses health risk on patients but also impact occupational safety in health-care settings. The need to control bioaerosols in indoor environments has led to numerous strategies and technologies in the past decades. Therefore, an increasing number of air-cleaning technologies are being adopted to remove indoor bioaerosols. Currently, bioaerosol removal approaches include electret filtration (Yang and Lee, 2005; Yang *et al.*, 2007), electrostatic precipitation (Li and Wen, 2003), ozone (Li and wang, 2003), ultraviolet germicidal irradiation (Lin and Li, 2002; Lee, 2011; Park *et al*., 2012), photocatalytic oxidation (Lin and Li, 2003), negative ions (Yu *et al.*, 2006), Plasma technology (Yang *et al*., 2011).

Among the technologies, the generation and dissemination of chlorine-related chemical disinfectant, such as gaseous chlorine dioxide (ClO_2), was viewed as an effective measure to neutralize surface and airborne bacterial particles. Although chlorine dioxide is a strong and broad spectrum disinfectant, it may cause health risk on skin, eye and respiratory tract under high concentration. According to The Occupational Safety and Health Administration (OSHA) of the USA, the workplace 8 hrs time-weight average (TWA) of chlorine dioxide should below 0.3 mg/m^3 (equivalent 0.1 ppm). The 15-min short term exposure limit of chlorine dioxide should below 0.9 mg/m^3 (equivalent 0.3 ppm) (OSHA. 2008). Gaseous chlorine dioxide is more useful under dedicated delivery, en-closed ventilation, personnel-absent field such as fumigation in serious biological contaminated building.

Membrane-less Electrolyzed water (MLEW) is generated by electrolysis of saline brine in a container within anodic and cathodic electrodes without separating membrane (Clark

* Corresponding author. Tel.: +886-5-3622889 ext. 841
E-mail address: shinhaoyang@ntu.edu.tw

and Barrrett, 2006; Kohno *et al.*, 2007; Park *et al.*, 2007; Guentzel *et al.*, 2008; Huang *et al.*, 2008). Its near-neutral pH product contains high oxidation-reduction potential (>1,000 mV) and free available chlorine (FAC, measuring the concentration of Cl_2, HOCl, OCl^- in liquid form) compounds. The MLEW has been reported to pose antimicrobial reactions against a variety of microorganisms in food industry and considered as alternative of traditional disinfectants. The bactericidal effect of MLEW on metal and glass surface against *E.coli* O157:H7, *Salmonella enteritidis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* has been performed in previous studies (Deza *et al.*, 2005; Abadias *et al.*, 2008). Methicillin-resistant *S. aureus* (MRSA) and *Acinetobacter baumannii* were reduced with $10^{6.8}$ fold by electrolyzed water fogging treatment while tested on ceramic tiles (Clark *et al.*, 2007). Besides, according to the previous investigations (Guentzel *et al.*, 2008; Arevalos-Sánchez *et al.*, 2012; McCarthy and Burkhardt, 2012), the electrolyzed water gains the advantages of non-irritating response of mucous membranes and skin. In addition, the electrolyzed water also reveals less toxicity and environmental impact than other chemical disinfectants. Up to FAC 200 ppm of MLEW was considered as safe level (Park *et al.*, 2007). Despite of widely used and proven effectiveness of against surface contamination, the MLEW has not been studied for the capacity to disinfectant the bioaerosols. The objective of the study is evaluating the inactivation efficiency of MLEW on bioaerosols in indoor environment, using environmental controlled chamber and cultured-based assay to determine the dose-response relationship among species, active concentration and ventilation rate.

THEORY

The decay curves of bioaerosol concentration were more difficult to compare than the rates of bioaerosol inactivation. Therefore, bioaerosol concentration decay was analyzing using the following equations:

$$dC/dt = -kC, \quad (1)$$

$$C_t = C_0 \exp(-kt),\ k = k_n \text{ or } k_a, \quad (2)$$

where C is the bioaeroosl concentration (CFU/m^3); C_0 and C_t are the initial concentration of target bioaerosols and the concentration thereof at time t, respectively (CFU/m^3); t is the residence time (min); k is the decay coefficient of bioaerosol concentration (min^{-1}); and, k_n and k_a are the decay coefficients of bioaerosol concentration associated with natural decay (physical removal) and MLEW spraying (physical removal and chemical MLEW inactivating, equal to the total eliminating), respectively (min^{-1}). The coefficients of C_0, C_t and t were measured in each experiment. The decay coefficient (k) is a regression coefficient in an exponential regression analysis, specified by Eq. (2). The subscripts of k_n and k_a refer to natural decay and MLEW spraying, respectively. According to the definition of k_a and k_n, the decay coefficient of the k_{ai} (only chemical MLEW inactivating) is defined as the following equation,

$$k_{ai} = k_a - k_n, \quad (3)$$

METHODS

Generation of Membrane-less Electrolyzed Water

The MLEW used in the study was generated by hand-made electrolyzing device. The schematic diagram of electrolyzing device is shown in Fig. 1. The device consists of 850 mL plastic container filled with saturated NaCl solution (6.15 M). Two 10 cm × 2 cm Pt/Ti base electrodes module was set inside the container as cathode and anode with the gap of 0.8 cm between electrodes. The current density is 25 Amp/dm^2 (ampere per decimeters, ASD) in the electrolysis container. With 30 minutes of electrolyzing process, the FAC concentration of NaCl solution would rise up to over 10,000 ppm. This 850 mL solution with high FAC concentration was then diluted with deionlized water (Milli-Q, Millipore, Billerica, MA, USA) to FAC 50 and 100 ppm as the ready-to-spray MLEW disinfectant. According to the study (Park *et al.* 2007), the FAC of MLEW up to 200 ppm was considered as safe level and tested to inactivate Norovirus.

The FAC concentration of the MLEW was quantified following the N, N-dimethyl-p-phenylenediamine (DPD) colorimetric method, using portable spectrometer (DR 2800, HACH, Loveland, CO, USA). The pH of the MLEW was measured using pH meter (CyberScan pH 510, Eutech, Inc., Singapore). The MLEW was disinfectant was subsequently pumped and sprayed with70 kg/cm^2 through 4 μm orifice diameter (No. 4) and 8 μm orifice diameter (No. 8) nozzles into the test chamber (shown in Fig. 2 and Fig. 3).

Bioaerosol Preparation

Staphylococcus aureus (*S. aureus*, ATCC 6538) bioaerosols was chosen as the tested aerosols. Three *S.*

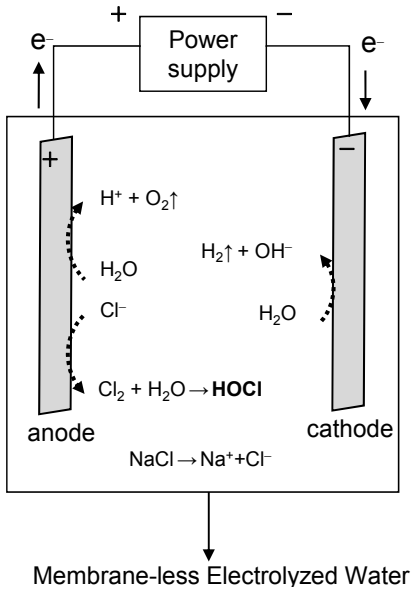

Fig. 1. The schematic diagram of electrolyzing device.

aureus colonies were extracted from agar plate cultures to a conical flask containing 30 mL tryptic soy broth (TSB, Bacto™) with a loop. The TSB culture was then shaken at 85 rpm for 16–24 h at a temperature of 37°C. Following incubation, the TSB culture was centrifuged at 2,500 rpm for 5 mins. The supernatant was then removed, 30 mL of PBS solution (phosphate buffered saline, pH 7.2) were added and the *S. aureus* sediments were resuspended. Then, the osmotic pressure between the microbial cellular fluids and the buffer solution was minimized using PBS buffer solution. The above steps (except for incubation) were repeated twice to remove the TSB medium. The final PBS solution (*S. aureus* stock) was used for the bioaerosol generation. The concentration of viable *S. aureus* in the PBS solution was determined by counting colony-forming units (CFUs) on agar plates.The test bioaerosols were generated by using a Collison three-jet nebulizer (BGI Inc., Waltham, MA). The generated bioaerosols were dried by the diffusion dryer. The dried bioaerosols then passed through a Krypton 85 (Kr-85, model 3077, TSI Inc.) radioactive source, which neutralized them to the Boltzmann charge equilibrium. After it had passed through the neutralizer, the tested aerosol was delivered into testing chamber to determine initial concentration. The airborne *S. aureus* bacterial concentration was measured by an Andersen single-stage impactor sampler (Andersen Samplers, Inc., Atlanta, GA, USA) supplied with TSA plates (BBL Trypticase™ Soy Agar, BD, NJ, USA). The Anderson single-stage impactor has a 50% cut point size of 0.58 μm. The impactor was recommended by American Conference of Governmental Industrial Hygienists (ACGIH, 1999) and Taiwan Environmental Protection Agency (Taiwan EPA, 2008) to collect viable bacteria aerosols. The sampling flow rate is about 28.3 L/min. The each sampling time is 30 seconds.

A λ virus phage (BCRC 70193) was selected as the model strain of viruses. The λ virus phage is a harmless virus with an isometric head of approximately 0.05 μm in diameter, a thin flexible tail of approximately 0.15 μm in length, and has a diameter of approximately 7 nm. The host of the λ virus phage is *E. coli* K12S (BCRC 14894), and the phage was evaluated as a prospective surrogate for water-borne and food-borne viruses. 1 mL of a 16 hours *E. coli* K12S (as host cell) TSB culture was placed into each TSA plate and laid aside for 2 hours (each experimental set required six plates). The redundant *E. coli* K12S TSB culture was removed from the surface of the TSA plates, and 100 μL of viral stock were added to each plate. A sterile bent glass rod was used to spread the host culture and the λ virus phage stock uniformly over the surface of the agar plates. All plates were incubated for 8–16 h at a temperature of 37°C. Following incubation, 6 mL of sterilized distilled water were placed into each plate, and the plates were shaken at 50–60 rpm for 5 min. The supernatant was centrifuged at 10,000 rpm for 10 min and filtered with a 0.22 μm Millex GS filter unit (Millipore Corporation, Carrigtwohill, Co. Cork, Ireland) to remove the host cells. The filtered liquid (λ virus phage stock) generated the bioaerosols to determine initial concentration. Finally, the infective λ virus phage concentration in the stock was enumerated by counting the plaque-forming unit (PFU) (using the double layer agar method). An AGI-30 sampler (Model 7540, ACE GLASS Inc., NJ, USA) and a sampling pump (All Field Tech, Taiwan) were used for the λ virus bioaerosols sampling. This sampler was recommended by the American Conference of Governmental Industrial Hygienists and the International Aerobiology Symposium for sampling viable microorganisms (Jensen *et al.*, 1992), and its collection efficiency for aerosolized viruses was comparable to those of other samplers (Tseng and Li, 2005). The collection efficiency of the sampler is a function of the particle diameter and the sampling flow rate (Hogan *et al.*, 2005). The sampling flow rate was 12.5 L/min and the sampling time was 6–8 min. Sterilized distilled water was used for sampling the aerosolized λ virus phage (Tseng and Li, 2005; Yu *et al.*, 2008).

Experimental Set Up

Fig. 2 schematically depicts the experimental setup for the aerosol inactivation experiment. The experimental setup comprises an aerosols nebulizer, charge neutralizer, makeup air device, MLEW spraying device (see Fig. 3), bioaerosol impactor or impinger. The model bacterial strains were aerosolized from suspension into the chamber by a three-jet Collison nebulizer (BGI Inc., Waltham, MA), operated at a flow rate of 2.5 L/min. The bacterial aerosol was dried by the diffusion dryer. The dried aerosol then passed through a Kr-85 radioactive source, which neutralized them to the Boltzmann charge equilibrium. After passing through the neutralizer, the aerosol was delivered into the stainless-steel test chamber ($80 \times 80 \times 80$ cm^3). Similar with indoor environment, bacterial aerosols in the test chamber may be easily diluted and removed by increasing fresh air intake, result in the decay of airborne concentration. To clarify the "natural ventilation decay" effect of fresh air intake rather than disinfectant intervention, several ventilating experimental parameters of the test chamber were examined in the study.

Before experiments started, the chamber was purged and well stabilized by clean air (without aerosols; which was produced by using HEPA filtrated air) to let the inside aerosol concentration was nearly zero. Two fans and a pump were utilized to retain a stable airflow and control the number of air exchanged rate per hour (ACH, h^{-1}). In this chamber system, the ACH is equal to the total airflow per volume of the tested chamber. HEPA filtrated clean air was employed as makeup air. At a fixed total airflow rate, clean air rate was changed to survey its effect on the decay behavior of bioaerosols. Two total ACH parameters, 0.5 and 1.0 h^{-1}, were set in experiment. The initial relative humidity inside the chamber was set at 30% by changing the ratio of flow rate of a dry gas stream to that of a humidified gas stream generated by a water vapor saturator. The relative humidity was monitored with Q-trak (Model 8550, TSI Inc., USA). The MLEW sprayed droplets were measured by the Scanning Mobility Particle Seizer (SMPS, Model 3934 TSI Inc.) in the testing chamber. The MLEW sprayed droplet diameters (CMD) from the No. 4 and No. 8 nozzles were about 0.12 and 0.2 μm. The main active chemical principal

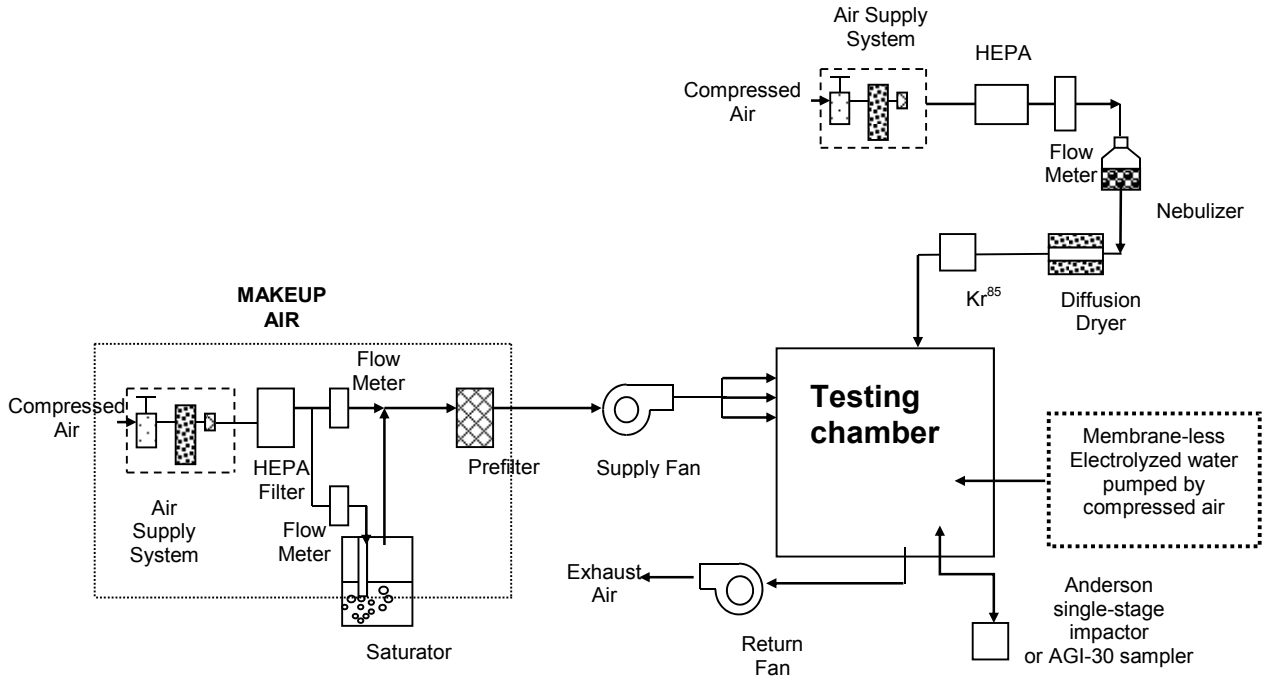

Fig. 2. Schematic diagram of experimental setup.

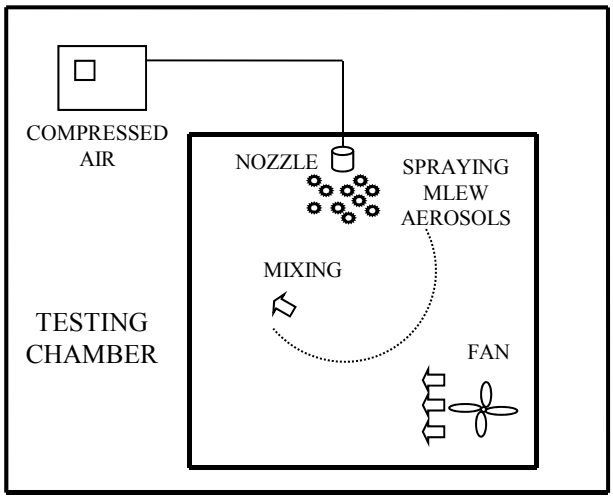

Fig. 3. Schematic diagram of MLEW spraying system.

of the MLEW is hypochlorous acid (HOCl) molecule, which is only presented in liquid phase. HOCl converts to Cl_2 once the droplet is evaporated after sprayed:

$$2HClO_{(aq)} + 2H^+ + 2e^- \rightarrow Cl_{2(g)} + 2H_2O$$

In the study, we keep the high relative humidity (> 90%) inside the test chamber after MLEW spraying. The MLEW droplet can remain liquid to clarify its microbicidal efficiency.

To determining the initial airborne bioaerosol concentration, the time-concentration calibration curve of test chamber was established by continuously deliver bacterial and virus aerosols and collect samples in 30 minute interval. The natural decay constant (k_n) was defined as the first-order decay constant (k_a) of bioaerosols concentrations without using the MLEW disinfectant spraying among various ACH parameters setting in the chamber.

RESULTS AND DISSCUSSIONS

The Calibration Curve of Bacterial Aerosols in Test Chamber

Fig. 4 shows the calibration curve for bacterial aerosol concentration along with continuously delivery of the test chamber. The linear relationship between bacterial concentration and delivery time can be observed. For *S. aureus*, the aerosol concentration can reach to about 3×10^4 CFU/m^3 after 50 minutes delivery. In addition, the concentration of *λ virus* aerosol also reached about 3×10^4 PFU/m^3 after 60 minutes delivery. Hence, the subsequent air exchanged rates (ACH 0.5 and 1.0 h^{-1}) natural decay and MLEW inactivation experiments all applied the initial bacterial aerosols concentration at 3×10^4 CFU/m^3 and initial *λ* viral aerosols concentration at 3×10^4 PFU/m^3

The Natural Decay for Bioaerosols

The bioaerosol removal effect caused by increasing ACH in the test chamber was shown in Fig. 5 and Fig. 6. Under total ACH of test chamber were set at 0, 0.5 and 1.0 h^{-1}, the k_n values of *S. aureus* bioaerosol were 0.015, 0.091 and 0.145 (min^{-1}), respectively. The result indicated increasing fresh air intake results in the obviously removal effect of airborne bioaerosols. For the *λ virus* aerosol, the the k_n values were 0.013, 0.081 and 0.138 (min^{-1}) under ACH of the test chamber were set at 0, 0.5 and 1.0 h^{-1}, respectively. These data also performed the same trend found on natural decay effect of *S. aureus* aerosol. Besides, the relatively low natural decay constant (k_n = 0.015 and 0.013) of both

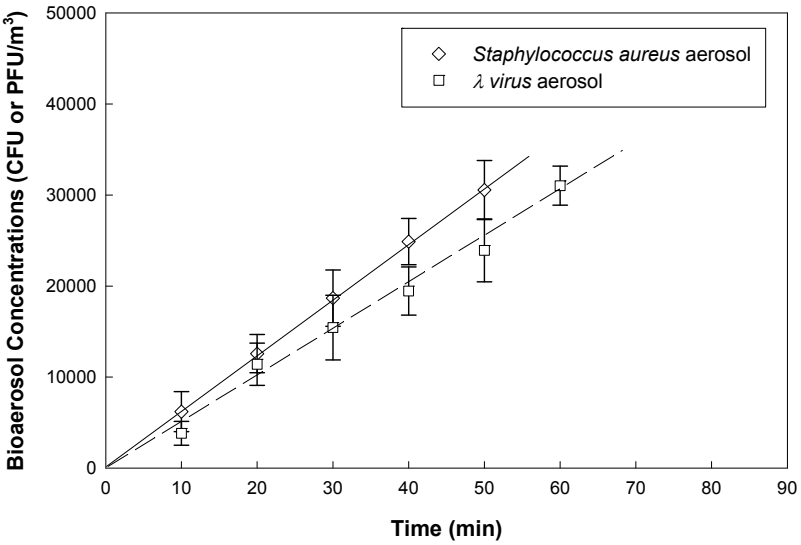

Fig. 4. Calibration curve of bioaerosols delivery in the test chamber.

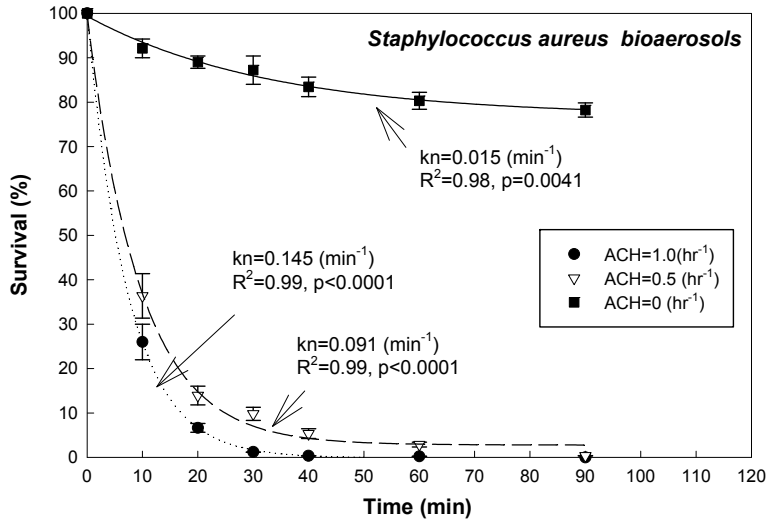

Fig. 5. Natural ventilation decay of *Staphylococcus aureus* aerosol in the test chamber.

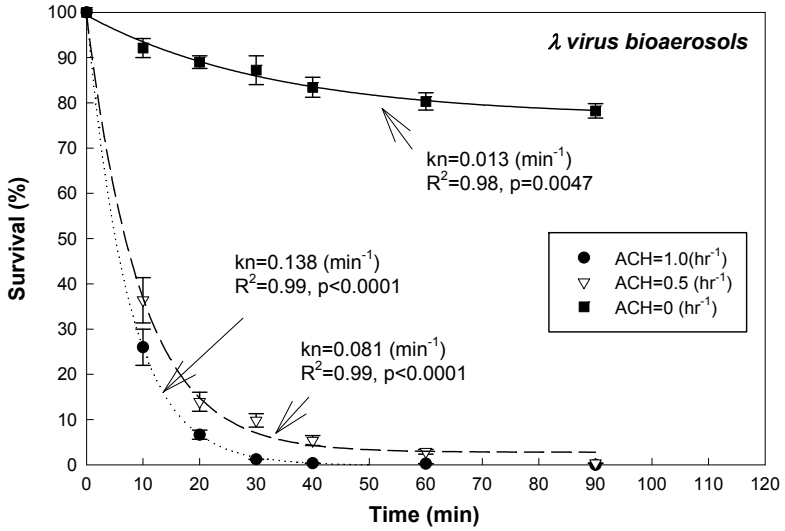

Fig. 6. Natural ventilation decay of λ *virus* aerosol in the test chamber.

bioaerosol under ACH = 0 hr^{-1} indicated the gravity deposition, wall loss and desiccation of aerosols were not significant in the test chamber. In the experiment of natural decay of our study, the initial relative humidity inside the chamber was set as low as 30%. Previous studies related to environmental factors suggest that the transmission efficiency and viability of viral bioaerosols decreased with increasing relative humidity (Pica and Bouvier, 2012). In general, over 90% of the bioaerosol could remain airborne for 30 minutes after delivering into test chamber. The bioaerosols concentrations were fulfilled to conduct next step of natural ventilation decay and MLEW inactivation.

The Inactivation Efficiency of MLEW Spray Against Bioaerosol

Fig. 7 shows elimination efficiency of *S. aureus* aerosol using FAC 100 ppm MLEW, sprayed with No. 8 nozzle, at ACH of 0.5 and 1.0 hr^{-1}. The k_a values for FAC 100 ppm MLEW sprayed with No. 8 nozzle at ACH of 0.5 and 1.0 hr^{-1} against *S. aureus* were 0.512 and 0.554 (min^{-1}), respectively. Compare to the k_n value under the same parameter without MLEW intervention (k_n = 0.091 and 0.145 at ACH of 0.5 and 1.0 h^{-1}), the spray of MLEW perform the effective inactivating effect against *S. aureus* aerosol. And according to the Eq. (3), the k_{ni} (inactivation coefficient) for FAC 100 ppm MLEW sprayed with No. 8 nozzle at ACH of 0.5 and 1.0 hr^{-1} against *S. aureus* were 0.421 and 409 (min^{-1}).

Fig. 8 shows elimination efficiency of *λ virus* aerosol using FAC 100 ppm MLEW, sprayed with No. 8 nozzle, at ACH of 0.5 and 1.0 hr^{-1}. The k_a values for FAC 100 ppm MLEW sprayed with No. 8 nozzle at ACH of 0.5 and 1.0 hr^{-1} against *λ virus* were 0.493 and 0.541 (min^{-1}), respectively. Compare to the k_n value under the same

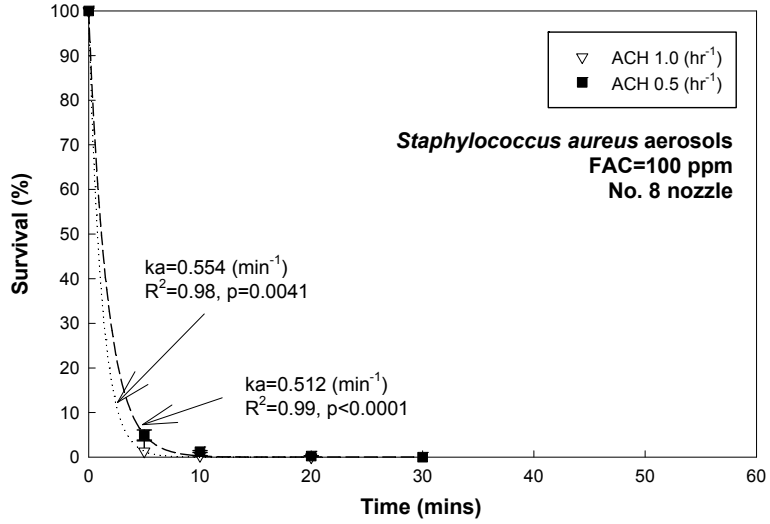

Fig. 7. Inactivation efficiency of *Staphylococcus aureus* aerosols using FAC 100 ppm MLEW at ACH of 0.5 and 1.0 (hr^{-1}), sprayed with No. 8 nozzle in the test chamber.

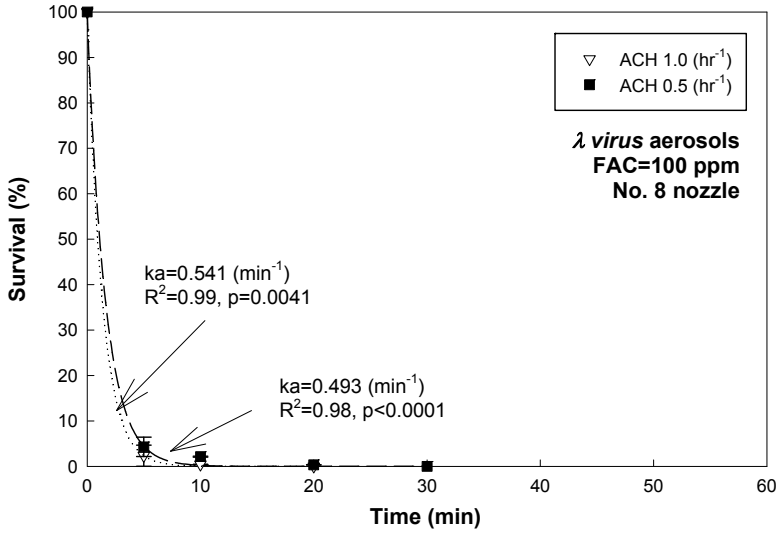

Fig. 8. Inactivation efficiency of *λ virus* aerosols using FAC 100 ppm MLEW at ACH of 0.5 and 1.0 (hr^{-1}), sprayed with No. 8 nozzle in the test chamber.

parameter without MLEW intervention (k_n = 0.081 and 0.138 at ACH of 0.5 and 1.0 h^{-1}), the spray of MLEW perform the effective inactivating effect against *λ virus* aerosol. And according to the Eq. (3), the k_{ni} for FAC 100 ppm MLEW sprayed with No.8 nozzle at ACH of 0.5 and 1.0 hr^{-1} against *λ virus* were 0.412 and 403 (min^{-1}).

For both ACH of 0.5 and 1.0 hr^{-1} application, the concentration of *S. aureus* and *λ virus* aerosol decreased from 3×10^4 to 0 CFU/m^3 and 3×10^4 to 0 PFU/m^3 after 20 minutes. The result indicated MLEW spray can still perform the airborne biological contamination inactivating capacity even under higher ventilation rate. According to the experimental data, it is also finding that the k_n value under the testing parameters increased with the ACH. When total ACH was increased, the air exchange rate in the testing chamber increased. High air exchange resulted in significant bioaerosols decay.

Effect of FAC on the Inactivation Efficiency

Fig. 9 shows elimination efficiency of *S. aureus* aerosol using FAC 50 and 100 ppm MLEW, sprayed with the No. 8 nozzle and ACH = 1.0 h^{-1}. The inactivating constant k_a value of FAC 50 ppm and 100 ppm were 0.481 and 0.554 (min^{-1}), respectively. According to the Eq. (3), the k_{ai} value of FAC 50 ppm and 100 ppm were 0.336 and 0.409 (min^{-1}) (k_n for ACH = 1.0 h^{-1} is 0.145 min^{-1}). Similarly, The inactivating constant k_a value of *S. aureus* aerosol using FAC 50 and 100 ppm MLEW, sprayed with the same No. 4 nozzle and ACH = 1.0 h^{-1} were 0.422 and 0.453 (min^{-1}). According to the Eq. (3), the k_{ai} value of FAC 50 ppm and 100 ppm were 0.277and 0.308 (min^{-1}).

Fig. 10 displays elimination efficiency of *λ virus* aerosol using FAC 50 and 100 ppm MLEW, sprayed with the same No. 8 nozzle and ACH = 1.0 h^{-1}. The inactivating constant k_a value of FAC 50 ppm and 100 ppm were 0.492 and

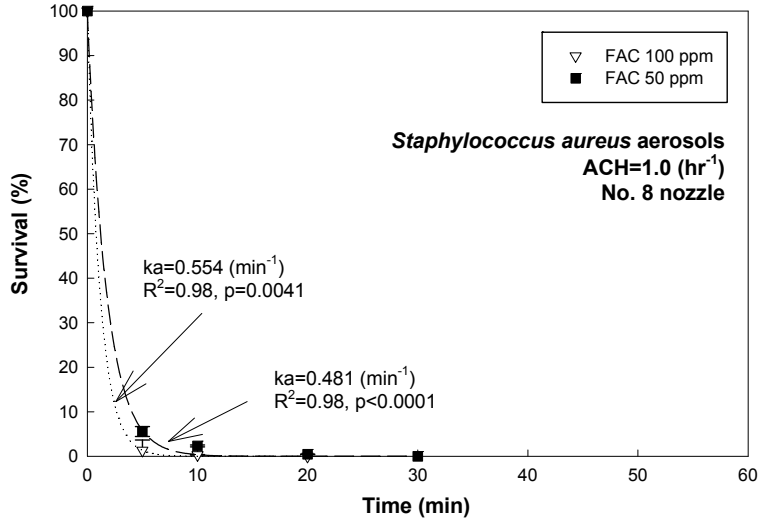

Fig. 9. Inactivation efficiency of *Staphylococcus aureus* aerosols using FAC 50 and 100 ppm MLEW , sprayed with No. 8 nozzle in the test chamber (ACH = 1.0 h^{-1}).

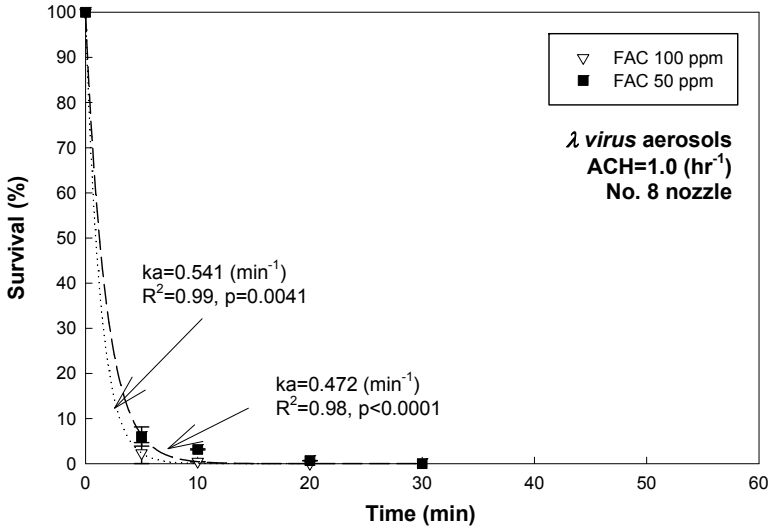

Fig. 10. Inactivation efficiency of *λ virus* aerosol using FAC 100 and 200 ppm MLEW , sprayed with No. 8 nozzle in the test chamber (ACH = 1.0 h^{-1}).

0.541 (min^{-1}), respectively. According to the Eq. (3), the k_{ai} value of FAC 50 ppm and 100 ppm were 0.354 and 0.403 (min^{-1}) (k_n for ACH = 1.0 h^{-1} is 0.138 min^{-1}). Similarly, The elimination constant k_a value of *λ virus* aerosol using FAC 50 and 100 ppm MLEW, sprayed with the same No. 4 nozzle and ACH = 1.0 h^{-1} were 0.410 and 0.439 (min^{-1}). According to the Eq. (3), the k_{ai} value of FAC 50 ppm and 100 ppm were 0.282 and 0.301 (min^{-1}). As predicted, lower inactivating efficiency was followed with lower initial FAC concentration of MLEW. The higher initial FAC concentration of MLEW leads better inactivating efficiency against bioaerosols. These trends could also been confirmed in previous studies which use various kinds of electrolyzed water disinfectant against bacteria in test tubes and surface. In the study, the initial bacterial aerosols concentration was set at 3×10^4 CFU/m^3, which is heavy airborne contamination. Also, single spray mode was applied to understand the acting time and inactivating efficiency of MLEW under heave contamination condition. Multiple spray mode of MLEW could be applied to inactivate the robust microbes such as Gram positive bacteria and fungus in the future studies.

Effect of Spraying Nozzles on the Inactivation Efficiency

Fig. 11 shows inactivation efficiency of *S. aureus* aerosol using FAC 100 ppm MLEW, sprayed with No. 4 and No. 8 nozzles. The ACH of test chamber was set at 1.0 h^{-1}. The k_a values for FAC 100 ppm MLEW sprayed with No. 4 and No. 8 nozzles against *S. aureus* were 0.453 and 0.554 (min^{-1}), respectively. According to the Eq. (3), the k_{ai} value for FAC 100 ppm MLEW sprayed with No. 4 and No. 8 nozzles against *S. aureus* were 0.308 and 0.409 (min^{-1}). Fig. 12 indicates inactivation efficiency of *λ virus* aerosol using FAC 100 ppm MLEW, sprayed with No. 4 and No. 8 nozzles. The ACH of test chamber was set at 1.0 h^{-1}. The k_a values for FAC 100 ppm MLEW sprayed with No. 4 and No. 8 nozzles against *λ virus* were 0.439 and 0.541 (min^{-1}), respectively. According to the Eq. (3), the k_{ai} value for FAC 100 ppm MLEW sprayed with No. 4 and No. 8 nozzles against *λ virus* were 0.301 and 0.403 (min^{-1}). Focused in the difference of inactivating efficiency between applying No. 4 and No. 8 nozzles, better efficiency was followed with larger spray orifice diameter (No. 8 nozzle) could be found.

The result of inactivating experiment showed that spraying MLEW with No. 8 nozzle can yield better inactivating efficiency than No. 4 nozzle under the same initial FAC concentration. We sprayed FAC 50 ppm MLEW with No. 8 nozzle for *S. aureus* bioaerosols could yield k_{ai} of 0.336 (min^{-1}). Nevertheless, we raised FAC concentration double to 100 ppm but k_{ai} was 0.409 with No. 4 nozzle spraying. These results implicated orifice diameter has influent inactivation efficiency more than initial FAC concentration of MLEW while inactivating *S. aureus and λ virus aerosols*. Smaller orifice diameter is a mechanical disturbance that generate fine size droplet that accelerate the interfacial mass transfer of chlorine gas (Cl_2), result in appreciable chlorine loss. Park *et al.* (2007) reported approximately 70% of FAC concentration was lost and 1.3 ± 0.11 pH unit was increased during deliver hypochlorous acid solution with dynamic fogger to steel and ceramic surface. In this study, MLEW was pumped with70 kg/cm^2 through both No. 4 and No. 8 nozzle. In Hsu *et al.* (2004) study, the smaller sprayer orifice size produced higher reduction (spraying with orifice size 1.016 mm result in 86% chlorine reduction and 0.508 mm result in 95% chlorine reduction) in chlorine concentration than larger orifices size (1.499 mm result in 81% chlorine reduction) under the same pumping pressure (all under 103 KPa pumping) was found. And the larger droplet size would be generated from the larger orifices size. According to the experimental results, the MLEW sprayed droplet diameters (CMD) from the No. 4 and No. 8 nozzles were about 0.12 and 0.2 μm. And, the MLEW disinfectant was subsequently sprayed through 4 μm orifice diameter (No. 4) and 8 μm orifice diameter (No. 8) nozzles.) The lower inactivation efficiency while using No. 4 nozzle might result from higher reduction of FAC concentration, which is the active antimicrobial principle of MLEW. However,

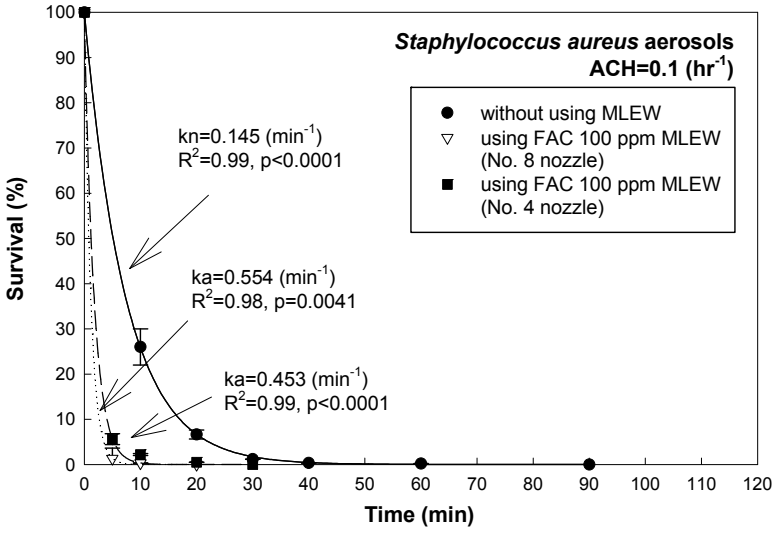

Fig. 11. Inactivation efficiency of *Staphylococcus aureus* aerosol using FAC 100 ppm MLEW, sprayed with No. 4 and No. 8 nozzles in the test chamber (ACH = 1.0 h^{-1}).

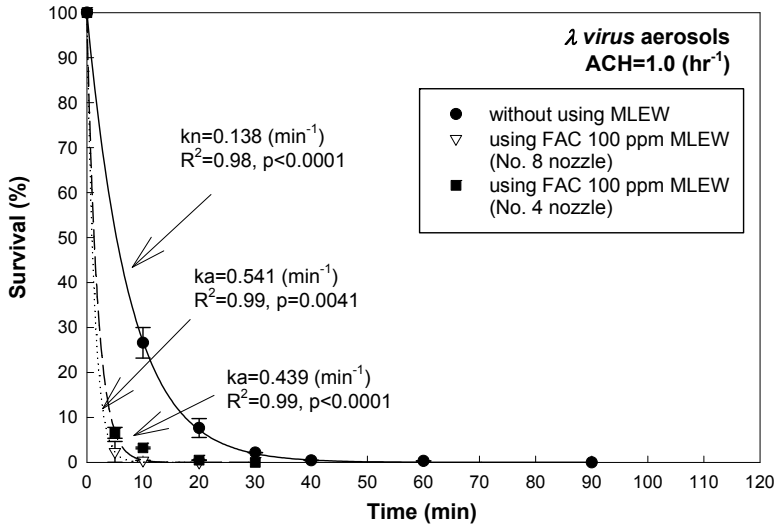

Fig. 12. Inactivation efficiency of *λ virus* aerosol using FAC 100 ppm MLEW, sprayed with No. 4 and No. 8 nozzles in the test chamber (ACH = 1.0 h^{-1}).

enclose test chamber was used to evaluate the inactivation efficiency against bioaerosols in the study. The methodology for collecting mist type of after-spray MLEW droplet to measure the pH and FAC value is needed in future research.

CONCLUSIONS

Experimental results demonstrate that the MLEW effectively inactivate *S. aureus and λ virus* bioaerosols from environments controlled by testing chambers. The natural decay constant (k_n) of bioaerosols depends on the ACH. The decay constant (k_a) of bioaerosols using the MLEW increases as ACH, FAC and spraying nozzle size. The inactivation capability of the MLEW was confirmed as having a major effect on bioaerosol inactivation.

ACKNOWLEDGMENTS

The authors would like to thank the Institute of Occupational Safety and Health of Republic of China for financially supporting this research under Contract No. IOSH98-H313.

REFERENCES

Abadias, M., Usall, J., Oliveira, M., Alegre, I. and Vinas, I. (2008). Efficacy of Neutral Electrolyzed Water (NEW) for Reducing Microbial Contamination on Minimally-processed Vegetables. *Int. J. Food Microbiol.* 123: 151–158.

American Conference of Governmental Industrial Hygienists (ACGIH) (1999). Bioaerosols: Assessment and Control, Cincinnati.

Arevalos-Sánchez, M., Regalado, C., Martin, S.E., Domínguez-Domínguez, J. and García-Almendárez, B.E. (2012). Effect of Neutral Electrolyzed Water and Nisin on *Listeria monocytogenes* Biofilms, and on Listeriolysin O Activity. *Food Control* 24: 116–122.

Clark, J., Barrrett, S.P. and Stapleton, R. (2006). Efficiency of Super-Oxidized Water Fogging in Environmental Decontamination. *J. Hosp. Infect.* 64: 386–90.

Deza, M., Araujo, M. and Garrido, M. (2005). Inactivation of *Escherichia Coli*, *Listeria Monocytogenes*, *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* on Stainless Steel and Glass Surfaces by Neutral Electrolysed Water. *Lett. Appl. Microbiol.* 40: 341–346.

Eduard, W., Sandven, P. and Levy, F. (1993). Serum IgG Antibodies to Mold Spores in Two Norwegian Sawmill Populations: Relationship to Respiratory and Other Work-related Symptoms. *Am. J. Ind. Med.* 24: 207–222.

Guentzel, J.L., Lam, K.L., Callam, M.A., Emmons, S.A. and Dunham, V.L. (2008). Reduction of Bacteria on Spinach, Lettuce, and Surface in Food Service Areas Using Neutral Electrolyzed Oxidizing Water. *Food Microbiol.* 25: 36–41.

Hogan, C.J., Jr., Kettleson, E.M., Lee, M.H., Ramaswami, B., Angenent, L.T. and Biswas, P. (2005). Sampling Methodologies and Dosage Assessment Techniques for Submicrometre and Ultrafine Virus Aerosol Particles. *J. Appl. Microbiol.* 99: 1422–1434.

Hsu, S.Y., Kim, C., Hung, Y.C. and Prussia, S.E. (2004). Effect of Spraying on Chemical Properties and Bactericidal Efficacy of Electrolysed Oxidizing Water. *International J. Food Sci. Technol.* 39: 157–165.

Huang, Y.R., Hung, Y.C., Hsu, S.Y., Huang, Y.W. and Hwang, D.F. (2008). Application of Electrolyzed Water in the Food Microbiology. *Food Control* 19:329–45.

Jensen, P.A., Todd,W.F., Davis, G.N. and Scarpino, P.V. (1992). Evaluation of Eight Bioaerosol Samplers Challenged with Aerosols of Free Bacteria. *Am. Ind. Hyg. Assoc. J.* 53: 660–667.

Kohno, S., Kawata, T., Kaku, M., Fuita, T., Tsutsui, K., Ohtani, J., Tenjo, K., Motokawa, M., Tohma, Y. and Shigekawa, M. (2004). Bactericidal Effects of Acidic Electrolyzed Water on the Dental Unit Waterline. *Jpn. J. Infect. Dis.* 57:52–54.

Koskinen, O.M., Husman, T.M., Meklin, T.M. and Nevalainen, A.I. (1999). The Relationship between Moisture or Mould Observations in Houses and the State of Health of Their Occupants. *Eur. Respir. J.* 14: 1363–1367.

Lance, W. (1006). Indoor Particles: A Review. *J. Air Waste Manage. Assoc.* 46: 98–126.

Lee, B.U. (2011). Life Comes from the Air: A Short Review on Bioaerosol Control**.** *Aerosol Air Qual. Res*. 11: 921–927.

Lee, B.U., Hong, B.G., Lee, D.H., Chong, E.S., Jung, J.H., Lee, J.H., Kim, H.J. and Lee, I.S. (2012). Bacterial Bioaerosol Concentrations in Public Restroom Environments. *Aerosol Air Qual. Res*. 12: 251–255.

Li, C.S. and Wang, Y.C. (2003). Surface Germicidal Effects of Ozone for Microorganisms. *Am. Ind. Hyg. Assoc. J.* 64: 533–537.

Li, C.S. and Wen, Y.M. (2003). Control Effectiveness of Electrostatic Precipitation on Airborne Microorganisms. *Aerosol Sci. Technol.* 37: 933–938.

Lin, C.H. and Li, C.S. (2003). Effectiveness of Titanium Dioxide Photocatalyst Filters for Controlling Bioaerosol. *Aerosol Sci. Technol.* 37: 162–170.

Lin, C.Y. and Li, C.S. (2002). Control Effectiveness of Ultraviolet Germicidal Irradiation on Bioaerosols. *Aerosol Sci. Technol.* 36: 474–478.

McCarthy, S. and Burkhardt Iii, W. (2012). Efficacy of Electrolyzed Oxidizing Water Against *Listeria Monocytogenes* and *Morganella Morganii* on Conveyor Belt and Raw Fish Surfaces. *Food Control.* 24: 214–219.

Melbostad, E., Eduard, W., Skogstad, A., Sandven, P., Lassen, J., Sostrand, P. and Heldal, K. (1994). Exposure to Bacterial Aerosols and Work-related Symptoms in Sewage Workers. *Am. J. Ind. Med.* 25: 59–63.

Park, C.W., Yoon, K.Y., Byeon, J.H., Kim, K. and Hwang, J. (2012). Development of Rapid Assessment Method to Determine Bacterial Viability Based on Ultraviolet and Visible (UV-Vis) Spectroscopy Analysis Including Application to Bioaerosols**.** *Aerosol Air Qual. Res*. 12: 399–408.

Park, G.W., Boston, D.M., Kase, J.A., Sampson, M.N. and Sobsey, M.D. (2007). Evaluation of Liquid-and Fog-Based Application of Sterilox Hypochlorous Acid Solution for Surface Inactivation of Human Norovirus. *Appl. Environ. Microbiol.* 73: 4463–68.

Pica, N. and Bouvier, N.M. (2012). Environmental Factors Affecting the Transmission of Respiratory Viruses. *Curr. Opin Virol.* 2: 90–95.

Singh, U., Reponen, T., Cho, K.J., Grinshpun, S.A., Adhikari, A., Levin, L., Indugula, R. and Green, B.J. (2011). Airborne Endotoxin and β-D-glucan in PM_1 in Agricultural and Home Environments. *Aerosol Air Qual. Res*. 11: 376–386.

Taiwan Environmental Protection Agency (EPA) (2008). Guideline on Environmental Analysis Laboratory. NIEA E301.11C Available online at: http://www.niea.gov.tw/niea/LIVE/E30111C.htm

Tseng, C.C. and Li, C.S. (2005). Collection Efficiencies of Aerosol Samplers for Virus-Containing Aerosols. *J. Aerosol Sci.* 36: 593–607.

US Occupational Safety and Health Agency (OSHA). The Data is Available Online at: http://www.osha.gov/SLTC/healthguidelines/chlorinedioxide/recognition.html

Yang, S.H. and Lee, W.M.G. (2005). Filtration Characteristics of a Fibrous Filter Pretreated with Anionic Surfactants for Monodisperse Solid Aerosols. *J. Aerosol Sci.* 36: 419–437.

Yang, S.H., Lee, W.M.G., Huang, H.L., Luo, C.H., Huang, Y.C. and Wu, C.C. (2007). Removal of Bioaerosols in HVAC System Using the Electret Filters. *J. Harbin Inst. Technol.* (New Series) 14: 241–244.

Yang, S.H., Huang, Y.C., Luo, C.H., Lin, Y.C., Huang, J.W., Chuang, C.P.J., Chen, C.J., Fang, W. and Chuang, C.Y. (2011). Inactivation Efficiency of Bioaerosols Using Carbon Nanotube Plasma. *Clean* 39: 201–205.

Yu, K.P., Lee, G.W.M., Huang, W.H., Wu, C.C., Lou, C.I. and Yang, S. (2006). Effectiveness of Photocatalytic Filter for Removing Volatile Organic Compounds in the Heating, Ventilation, and Air Conditioning System *J. Air Waste Manage. Assoc.* 56: 666–674.

Yu, K.P., Lee, G.W.M., Lin, S.Y. and Huang, C.P. (2008). Removal of Bioaerosols by the Combination of a Photocatalytic Filter and Negative Air Ions. *J. Aerosol Sci.* 39: 377–392.

Received for review, May 11, 2012
Accepted, August 22, 2012