Effect of Dielectric Filter Discharge Plasma on the Inactivation of Aerosolized Pathogens Associated with Edible Insects

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ABSTRACT

The objective of this study was to determine the optimal disinfection treatment conditions of dielectric filter discharge (DFD) plasma against aerosolized pathogens relevant to edible insect-rearing environments. *Bacillus thuringiensis*, *Serratia marcescens*, and *Escherichia coli* O157:H7 are representative pathogens that may occur in edible insect-rearing environments. This study employed a treatment scheme in which plasma was applied during nebulization to treat aerosolized pathogens, after collection to treat filter-attached pathogens, or sequentially to both (overall pathogens). Negligible or minimal bactericidal effects were observed when DFD plasma was applied to aerosolized or filter-attached pathogens for 2 min. However, continuous treatment of both for 2 min each (a total of 4 min) resulted in significant inactivation, and longer treatment times (10 min) for filter-attached pathogens further enhanced the bactericidal effect (2.6~3.4 Log CFU/cm² reduction). No bactericidal effect was observed during storage after DFD plasma was turned off. Consequently, the highest bactericidal effect was obtained when DFD plasma was applied to aerosolized pathogens for 2 min, followed by an additional 10 min of treatment with filter-attached pathogens. These results indicate the potential use of DFD plasma for disinfecting aerosolized pathogens in edible insect-rearing environments.

Keywords: aerosolized pathogens; bacterial inactivation; cold plasma; edible insect

INTRODUCTION

The global population is expected to reach 9.8 billion by 2050, resulting in over a 70% increase in the demand for animal-based protein sources [1]. Edible insects rich in proteins, essential amino acids, and fatty acids are emerging as promising alternatives to conventional meat [2]. Additionally, rearing edible insects is more eco-friendly than livestock production because of their lower space and water requirements and greenhouse gas emissions. Accordingly, the number of edible insect farms is increasing worldwide [2].

In most edible insect farms, larvae are raised in enclosed spaces with high larval densities to maximize space efficiency [3]. However, this rearing process provides favorable conditions for pathogen proliferation and transmission, resulting in disease outbreaks and decreased productivity [3]. Pathogenic bacteria, such as *Bacillus thuringiensis* and *Serratia marcescens*, are lethal to Coleoptera and Lepidoptera, posing serious challenges during insect rearing. *S. marcescens* is pathogenic in humans [4]. *B. thuringiensis* has enterotoxin genes similar to those of *Bacillus cereus*, which may be associated with foodborne outbreaks [5]. Additionally, edible insects are reported to carry Shiga toxin-producing *E. coli*, including several pathogenic strains such as *E. coli* O157:H7, some of which cause foodborne illnesses [6]. Therefore, pathogenic bacteria that contaminate insect-rearing environments must be inactivated to ensure efficient insect production, protect human health, and enhance microbial safety during product consumption.

When living organisms, such as edible insects, are reared, pathogenic bacteria may not merely adhere to surfaces but can also exist in the air [7, 8]. Bacteria can be present in the air attached to airborne particles such as aerosols [9]. These aerosolized bacteria can be carried upward by convective air currents, and given their small size, they can persist in the air for a long time [9]. Bacteria can travel several meters from their origin, reach various surfaces, and pose the risk of secondary transmission [10]. To effectively prevent indoor transmission in edible insect farms, it is necessary to use an appropriate air disinfection system to inactivate pathogenic insect aerosols.

Various methods can be used to disinfect the air. Among non-chemical methods, filtration is the most commonly used. Various filters, such as high-efficiency particulate air (HEPA) and polypropylene melt-blown (PP) filters, are used to filter air in masks, medical devices, and air purifiers [11, 12]. However, such filters only capture aerosolized microorganisms on their surfaces without inactivating them, leading to the risk of secondary

transmission through ventilation systems [13]. Therefore, filtration methods must be used in combination with other disinfection methods.

Plasma is an emerging technology capable of disinfecting air and inactivating microorganisms when combined with various filters. Atmospheric plasma generates electromagnetic fields, UV photons, and reactive oxygen and nitrogen species (RONS), which play key roles in microbicidal activity [10]. Previous studies have shown that a plasma device coupled with a filter can remove and inactivate aerosolized microorganisms [14]. Baek et al. [10] developed a dielectric filter discharge (DFD) plasma device in which plasma was coupled with a PP filter and used to inactivate SARS-CoV-2 aerosols. However, most studies on plasma coupled with filter devices have focused on viral inactivation, and although some studies targeting bacteria have been reported, no studies have reported inactivation of pathogens that may occur during the rearing of edible insects. Additionally, it is necessary not only to evaluate the bactericidal effect of the plasma coupled with a filter device but also to identify the most effective processing conditions for bacterial inactivation.

The objective of this study was to evaluate optimal conditions for inactivating aerosolized pathogens related to edible insects (*B. thuringiensis*, *S. marcescens*, and *E. coli* O157:H7) using DFD plasma in which surface discharge occurs on a porous dielectric (air-passable) filter. The bactericidal effects were compared when pathogens were treated in the aerosolized state, after being attached to the filter, under continuous treatment of both states, and during storage time after plasma treatment.

MATERIALS AND METHODS

DFD Plasma Source

The DFD plasma source used in this study was identical to that described by Baek et al. [10]. The DFD plasma generator was designed for air permeability. Both sides of 3 mm zirconium oxide beads were covered with punched aluminum electrodes (0.5 mm thick, 2 mm punching hole diameter), forming a porous dielectric filter barrier.

Bacterial Strains and Microbial Preparation

B. thuringiensis (KACC 12061) and *S. marcescens* (KACC 11961) were cultivated in a 25 mL nutrient broth (MB cell, Seoul, Korea) at 30℃ for 24 h, respectively. *E. coli* O157:H7 (NCCP 17169) was cultivated in a 25 mL

tryptic soy broth (MB cell, Seoul, Korea) at 37℃ for 24 h. The cultures were washed as described by Yong et al. [15]. Finally, microbial pellets were resuspended in 25 mL sterile saline with optical density (600 nm) adjusted to 0.5. Final viable cell densities of the pathogens were approximately $10^{7\text{-}8}$ CFU/mL, respectively. Each pathogen suspension was prepared and aerosolized independently for all subsequent plasma treatments.

Generating and Treating Aerosols with DFD Plasma

The bacterial suspension was aerosolized using a nebulizer (HL100A; JOINMEDICAL, Seoul, Korea) to generate aerosolized pathogens. The nebulizer was connected to a closed cylindrical chamber equipped with a DFD plasma system (Fig. 1 (a)), as described by Baek et al. [10]. The pump was operated at 25 L/min during nebulization and then turned off. Aerosolized pathogens were passed through the plasma zone under a sinusoidal voltage of 1.24 kV at 8 kHz using ambient air at room temperature. Fig. 1 (b) shows voltage and current waveforms of the DFD plasma generator, illustrating typical dielectric barrier discharge (DBD) behavior. Pathogens were collected on a PP filter below the generator (99.95% efficiency for 0.3 μm particles), which was UV-sterilized for 30 min on both sides before use. HEPA filters were installed at the air inlet and outlet to prevent the release of pathogens. Control samples were prepared under identical conditions without plasma exposure.

Experimental Design of DFD Plasma Treatment

The experimental design of this study is illustrated in Fig. 2. In Experiment I, the bactericidal effects of DFD plasma were evaluated against aerosolized and filter-attached pathogens, and this evaluation was further extended to include continuous treatment with DFD plasma for both pathogen types (overall pathogens). For aerosolized pathogen treatment, plasma was operated during nebulization so that airborne bacteria were treated with DFD plasma for 2 min. For the filter-attached pathogen treatment, nebulization was performed without plasma operation; aerosolized pathogens were collected on the PP filter, and the filter-attached pathogens were subsequently treated with DFD plasma for 2 min. Thus, plasma exposure during nebulization targeted only aerosolized pathogens, whereas plasma exposure after nebulization targeted only filter-attached pathogens. For overall pathogen treatment, DFD plasma was applied for 2 min during nebulization and 2 min after nebulization to assess its continuous inactivation effect on both aerosolized and filter-attached pathogens (total treatment time: 4 min).

In Experiment II, the bactericidal effect on overall pathogens with different storage times after DFD plasma treatment was evaluated. DFD plasma was applied for 2 min during nebulization and for an additional 2 min after nebulization to treat the overall pathogens, after which the plasma was turned off. To assess residual bactericidal effect during storage after the plasma was turned off, the PP filter with attached pathogens was placed inside the device for 0 and 1 min. As shown in Fig. 3 (b), it takes 1 min for the ozone concentration inside the device to reach zero after the plasma is turned off; therefore, the maximum storage time was set to 1 min.

In Experiment III, DFD plasma was applied to aerosolized pathogens for 2 min, followed by additional treatments for 2, 5, and 10 min on filter-attached pathogens to evaluate bactericidal effects.

Microbial Analysis

After plasma treatment, each PP filter was vortexed with 20 mL of sterile saline for 2 min. The resulting suspension was serially diluted in saline. *B. thuringiensis* and *S. marcescens* were plated on nutrient agar, and *E. coli* O157:H7 was plated on tryptic soy agar (MB cell, Seoul, Korea). Aliquots (0.1 mL) were spread onto plates and incubated for 48 h at 30°C (*B. thuringiensis*, *S. marcescens*) or 37°C (*E. coli* O157:H7). The colony-forming units (CFU/cm²) were calculated as density using the following formula:

$$\text{Density} = \frac{\text{Avg. count}}{\text{Drop volume}} \times \text{Dilution} \times \text{Volume used to resuspend} \times \frac{1}{\text{Surface area}}$$

where Avg. count is the average of the raw data counts [CFU];

Drop volume is defined as the volume of the sample placed on the agar plate [mL]; Dilution is the $1/10^{-k}$, where k is an integer for 10-fold dilutions [no units];

Volume used for resuspension was the liquid volume required to resuspend the PP filter [mL];

Surface area is the resuspended surface area of the PP filter [cm²].

Optical Emission Spectroscopy and Ozone Concentration

The optical emission spectrum of the DFD plasma was recorded using a spectrometer (Maya 2000 PRO, Ocean Insight, Orlando, FL, USA) with an appropriate optical setup. The ozone concentration generated during plasma operation was monitored using a UV ozone analyzer (UV-106L; 2 B Technologies, Broomfield, CO, USA) in three stages: i) DFD plasma treatment of aerosolized pathogens, ii) DFD plasma treatment of filter-attached pathogens, and iii) post-treatment storage time. All ozone concentration generated during plasma operation was

measured under conditions without nebulization of the bacterial suspension.

Statistical Analysis

All data represent the mean values of three replicates. Statistical analysis was performed using one-way ANOVA in a completely randomized design with IBM SPSS software (v20.0; IBM SPSS Inc., Chicago, IL, USA). Significant differences among treatments were determined using Duncan's multiple range test ($p < 0.05$).

RESULTS AND DISCUSSION

Optical Emission Spectroscopy and Ozone Concentration

Optical emission spectroscopy was used to identify the reactive species generated by DFD plasma. As shown in Fig. 3 (a), the spectrum exhibited strong emission bands from molecular nitrogen (N_2) and its ionized form (N_2^+). The intense emission at 300–400 nm corresponds to the second positive system ($C^3\Pi_u$–$B^3\Pi_g$) of N_2, indicating excitation by energetic electrons, while the 380–470 nm region represents the first negative system ($B^2\Sigma_u^+$–$X^2\Sigma_g^+$) of N_2^+, reflecting plasma ionization. These results confirm that the DFD plasma operated in air produced excited N_2* and N_2^+ species, which serve as precursors for downstream RONS, such as O_3, $OH^{\cdot}$, and $ONOO^-$, that contribute to microbial inactivation and surface modification.

Fig. 3 (b) shows that ozone (O_3) was generated during the DFD plasma treatment time. DFD plasma generated approximately 0.5 ppm of ozone during the treatment of aerosolized pathogens, whereas the concentration increased to 6–7 ppm when treating filter-attached pathogens. After the plasma was turned off, the ozone level returned to zero within 1 min. In this experiment, it is presumed that the ozone generated during the nebulization of the bacterial suspension to produce aerosols was vented out by the operation of the pump. In contrast, when the DFD plasma was applied to the filter-attached pathogens, the pump was turned off, which likely increased the ozone concentration.

Experiment I: Bactericidal Effects of DFD Plasma on Aerosolized, Filter-Attached, and Overall Pathogens

Fig. 4 shows the bacterial inactivation of aerosolized, filter-attached, and overall pathogens treated with DFD plasma. When the bacterial suspension was nebulized to generate aerosols without DFD plasma treatment (control), the surviving populations of *B. thuringiensis*, *S. marcescens*, and *E. coli* O157:H7 were approximately

4.57, 6.19, and 5.04 Log CFU/cm^2, respectively. The populations of *B. thuringiensis*, *S. marcescens*, and *E. coli* O157:H7 in the aerosolized pathogens treated with DFD plasma did not differ significantly from those in the control group. The *B. thuringiensis* population was significantly decreased by 1.20 Log CFU/cm^2 compared with those in the control when the filter-attached pathogens were treated with DFD plasma, whereas the *S. marcescens* and *E. coli* O157:H7 populations were not significantly decreased compared with those in the control. However, when DFD plasma was continuously applied to both aerosolized and filter-attached pathogens (overall pathogens), the populations of *B. thuringiensis*, *S. marcescens*, and *E. coli* O157:H7 were significantly reduced by 1.24, 1.05, and 1.02 Log CFU/cm^2, respectively, compared with those in the control ($p < 0.05$).

In this study, aerosolized pathogens were generated by nebulizing a bacterial suspension, which remained moisture-containing. Accordingly, we hypothesized that DFD plasma treatment of these aerosols would generate RONS, thereby leading to effective bacterial inactivation. As confirmed by the optical emission spectroscopy results, the DFD plasma generated various nitrogen radicals (Fig. 3 (a)) that could react with water molecules to form additional RONS [16]. However, contrary to this expectation, bacterial inactivation was not observed under the present experimental conditions. This finding contrasts with our previous study, in which 300 s (5 min) of DFD plasma treatment during aerosolization of a SARS-CoV-2 suspension effectively inactivated 99.84% of the viral population [10]. Given that viruses lack a cell wall, they are generally more susceptible to disinfection, and the reactive species produced by the plasma can modify viral RNA, resulting in its inactivation [17]. Therefore, even with the same plasma device, the antimicrobial efficacy of the treatment may vary depending on the microorganisms' structural characteristics. Additionally, the ozone concentration generated by the DFD plasma during treatment of aerosolized pathogens was approximately 0.5 ppm (Fig. 3 (b)), which might be too low to achieve effective bacterial inactivation.

After nebulization, the aerosolized pathogens were attached to a PP filter located below the DFD plasma generator by pump operation. Therefore, in this study, the inactivation effects of DFD plasma were evaluated on pathogens attached to a filter. When filter-attached pathogens were treated with DFD plasma, a statistically significant reduction was observed only for *B. thuringiensis* under the present experimental conditions. The bactericidal effect of plasma is generally explained as being attributable to differences in the cell wall structures of bacteria. Gram-positive bacteria possess a thick peptidoglycan layer, whereas gram-negative bacteria have a thinner peptidoglycan layer and an additional outer membrane [15]. Given these structural differences, bacteria

often show varied responses to sterilization, including plasma treatment. In particular, Bende et al. [19] showed that the outer membrane of gram-negative bacteria serves as a more effective barrier against RONS than the peptidoglycan layer of gram-positive bacteria. Accordingly, several previous studies have suggested that gram-positive bacteria are more susceptible to inactivation by plasma treatment [18,19]. However, in contrast to these findings, some studies have reported opposing results, indicating that gram-negative bacteria may be more sensitive because the thick peptidoglycan layer of gram-positive bacteria confers greater resistance to chemical species [15]. Given the diverse results reported in previous studies, the present study could not clearly establish a definitive distinction in plasma susceptibility between gram-positive and gram-negative bacteria.

As shown in Fig. 4, the populations of *S. marcescens* and *E. coli* O157:H7 did not differ significantly from those in the control group, which may be attributed to the relatively low ozone concentration (approximately 6–7 ppm) generated during plasma treatment (Fig. 3 (b)). Supporting this, Gallagher et al. [20] reported that 28 ppm of ozone produced by plasma could inactivate 97% of *E. coli* in aerosols, confirming the ozone-dependent sterilization effect of plasma.

Interestingly, in the case of *S. marcescens* and *E. coli* O157:H7, no distinct bactericidal effects were observed when the DFD plasma was individually applied to aerosolized or filter-attached pathogens. However, when both steps were treated continuously (overall pathogens), a clearer bactericidal effect was observed, likely due to the increased total plasma exposure time. In addition, plasma treatment of aerosolized pathogens may leave some cells in a sub-lethally injured state, and these cells could become more susceptible to inactivation during the subsequent plasma exposure applied to filter-attached pathogens [21]. By contrast, for *B. thuringiensis*, some cells might already have been inactivated during the DFD treatment of filter-attached pathogens, which could explain the absence of an additional bactericidal effect under continuous plasma treatment. However, whether these mechanisms occurred in the present study remains uncertain and requires further verification.

In Experiment I, the DFD plasma treatment showed limited inactivation when applied individually to aerosolized or filter-attached pathogens. By contrast, continuous treatment resulted in noticeable bacterial reduction. However, since this effect was still limited, Experiment II was subsequently conducted to evaluate whether the bactericidal effect could be enhanced by applying a storage time.

Experiment II: Bactericidal Effect Depending on Storage Duration after DFD Plasma Treatment

In Experiment I, the highest bactericidal effect was observed when both aerosolized and filter-attached pathogens (overall pathogens) were continuously treated with DFD plasma. Therefore, these treatment conditions were selected for Experiment II. In Experiment II, 0 and 1 min of storage time were applied after turning off the DFD plasma to further enhance the bactericidal effect (Fig. 5).

The plasma device used in this study consists of a closed cylindrical chamber in which the inlet and outlet are fitted with HEPA filters, thereby limiting the loss of reactive species. Under these conditions, reactive species can remain even after the plasma is turned off, and these residual species are known to contribute to additional bacterial inactivation. Leipold et al. [22] inoculated *Listeria innocua* onto glass slides, which were placed in polyethylene bags and exposed to DBD plasma that was turned on and off. Ozone was preserved in a sealed bag for approximately 10 min after exposure to DBD plasma for 30 s, thereby increasing the inactivation of the inoculated *L. innocua.* Yong et al. [15] inoculated *E. coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes* onto agar plates and cheese slice samples. The samples were placed in plastic containers, exposed to DBD plasma, and left for up to 5 min with DBD plasma turned off. The results showed that more bacteria could be inactivated as the waiting time increased.

This study conducted a 1 min storage experiment to evaluate the bacterial inactivation capability of the residual ozone after turning off the DFD plasma. As shown in Fig. 3 (b), residual ozone is still present for 1 min even after the DFD plasma was turned off. Given that ozone is a representative reactive oxygen species with a relatively long lifetime, we expected that it would contribute to an additional bactericidal effect [23]. However, as shown in Fig. 5, no significant differences in bacterial inactivation were observed among the three species between 0 and 1 min of storage time. This can be attributed to the rapid decrease in the ozone concentration within 1 min of turning off the DFD plasma, resulting in an insufficient level of bacterial inactivation (Fig. 3 (b)). Therefore, storage for 1 min in an environment with decreasing ozone did not have any noticeable bactericidal effects on the tested bacteria.

Experiment III: Bactericidal Effects of DFD Plasma According to the Treatment Time for Overall Pathogens

In Fig. 6, the effect of bacterial inactivation over time was shown by varying the DFD plasma discharge time for filter-attached pathogens during the continuous treatment of aerosolized and filter-attached pathogens (overall

pathogens). The inactivation of all three bacterial species increased linearly with increasing plasma treatment time. The population of *B. thuringiensis* was reduced by 1.24, 1.89, and 3.41 Log CFU/cm^2 at treatment times of 2, 5, and 10 min, respectively. The population of *S. marcescens* decreased, with reductions of 1.05, 1.84, and 2.67 Log CFU/cm^2 at 2, 5, and 10 min, respectively. Also, the population of *E. coli* O157:H7 decreased by 1.02, 1.81, and 2.94 Log CFU/cm^2 at the same treatment times, respectively. This implies that the bactericidal effect of DFD plasma was significantly enhanced when the treatment time of filter-attached pathogens was increased after applying DFD plasma to aerosolized pathogens for 2 min.

Concentration of aerosolized bacteria in indoor environments varies depending on environmental conditions, seasonal changes, and human occupancy, but it generally remains within the range of approximately 2–3 Log CFU/m^3 [24]. As shown in Fig. 6, under the conditions showing the greatest bactericidal effect, the reduction level ranged from approximately 2.6 to 3.4 Log CFU/cm^2. Thus, the DFD plasma treatment applied in this study was expected to show an effective inactivation effect against aerosolized bacteria.

This study represents a preliminary investigation demonstrating the potential application of DFD plasma in rearing environments such as edible insect cages. Therefore, this plasma device can be considered for installation in insect-rearing cages and for operation with air circulation using a pump. However, further studies are required to determine the practical applications of this device. In particular, since it is intended for use during the rearing of edible insects, its potential adverse effects on insects must be thoroughly evaluated. Ozone, a key reactive species in plasma sterilization [10], can inactivate aerosolized bacteria in rearing environments; however, its potential adverse effects on edible insects must be carefully evaluated before practical application. Studies on the effects of ozone on insects have shown that exposure to various combinations of ozone concentrations and durations, such as 2–3 days at 5 ppm or 1 h at 1800 ppm, can lead to insect mortality [25]. Although the ozone concentration used in the current study (approximately 7 ppm) did not cause insect mortality, further verification is required. Additionally, technologies for the effective management and application of ozone emissions should be developed, and the introduction of ozone catalysts should be considered to reduce ozone concentration [26].

Conclusions

This study evaluated the disinfection effects of DFD plasma on aerosolized pathogens associated with insect pathogenic and foodborne bacteria that may occur in edible insect-rearing environments and confirmed its

potential applicability. The results showed that various RONS, including ozone, were generated during the DFD plasma discharge. When DFD plasma was applied to aerosolized pathogens for 2 min or to filter-attached pathogens for 2 min, no bactericidal effects were observed. However, a significant inactivation effect was observed when both aerosolized and filter-attached pathogens were continuously treated with DFD plasma. Especially, the highest bactericidal effect was obtained when DFD plasma was applied to aerosolized pathogens for 2 min, followed by an additional 10 min of treatment with filter-attached pathogens. In this treatment, reductions of 3.41, 2.67, and 2.94 Log CFU/cm² were observed in *B. thuringiensis*, *S. marcescens*, and *E. coli* O157:H7, respectively.

Inactivation of aerosolized microorganisms in edible insect rearing environments is expected to play an important role in ensuring the productivity and safety of edible insect production. This approach may also contribute to reducing microbial contamination risks and improving hygiene management in insect-rearing systems. However, given that this study was conducted under simulated conditions, further validation is required to confirm whether similar effects can be achieved in actual insect-rearing systems.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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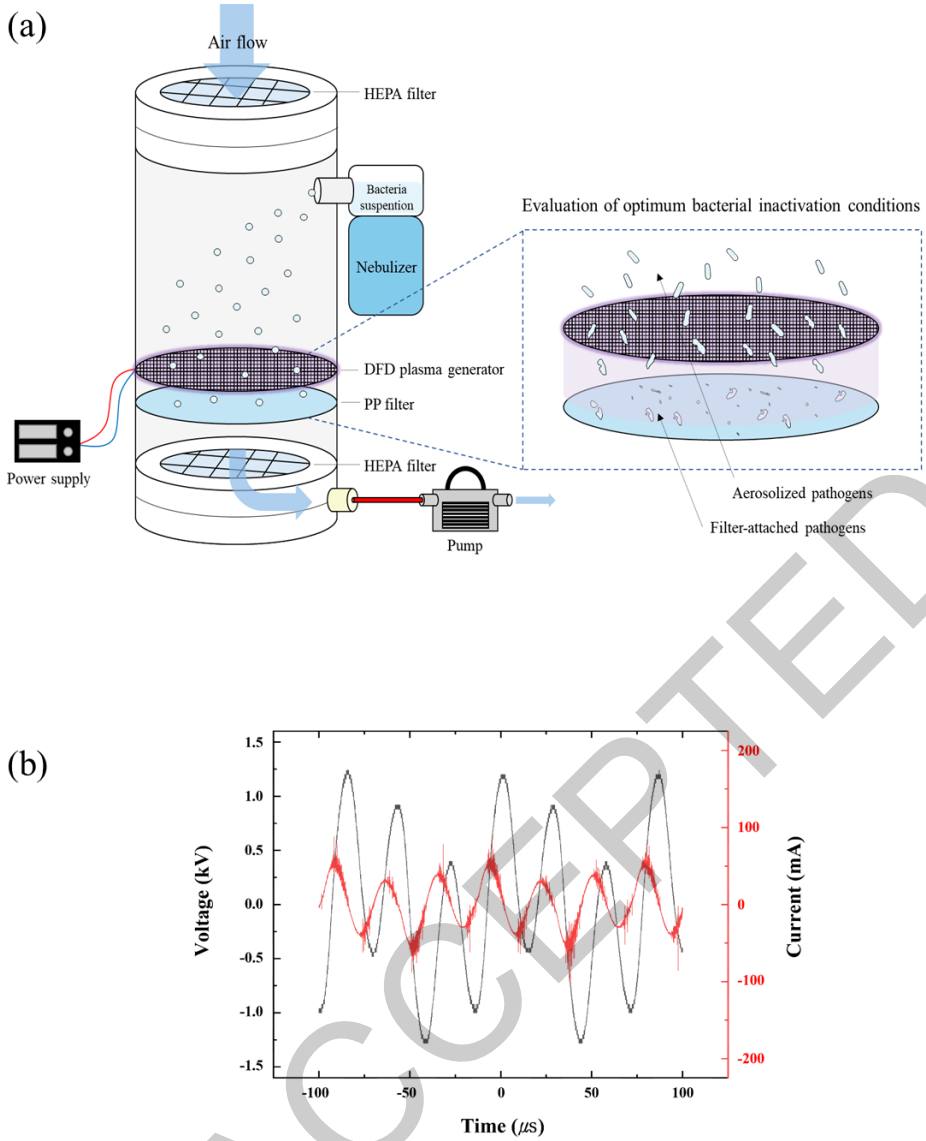
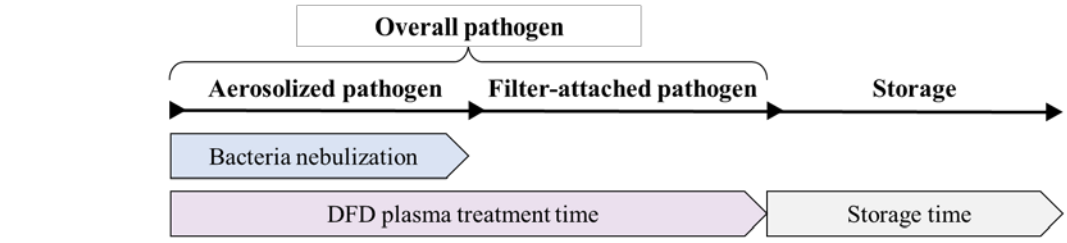


Fig. 1. (a) Schematic diagram of the dielectric filter discharge (DFD) plasma system for aerosolized pathogen inactivation and (b) **v**oltage and current waveforms during plasma treatment.



Experiment I	2 min	-	-
	-	2 min	-
	2 min	2 min	-
Experiment II	2 min	2 min	0 min
	2 min	2 min	1 min
Experiment III	2 min	2 min	-
	2 min	5 min	-
	2 min	10 min	-

Fig. 2. Diagram illustrating the DFD plasma treatment process in this study.

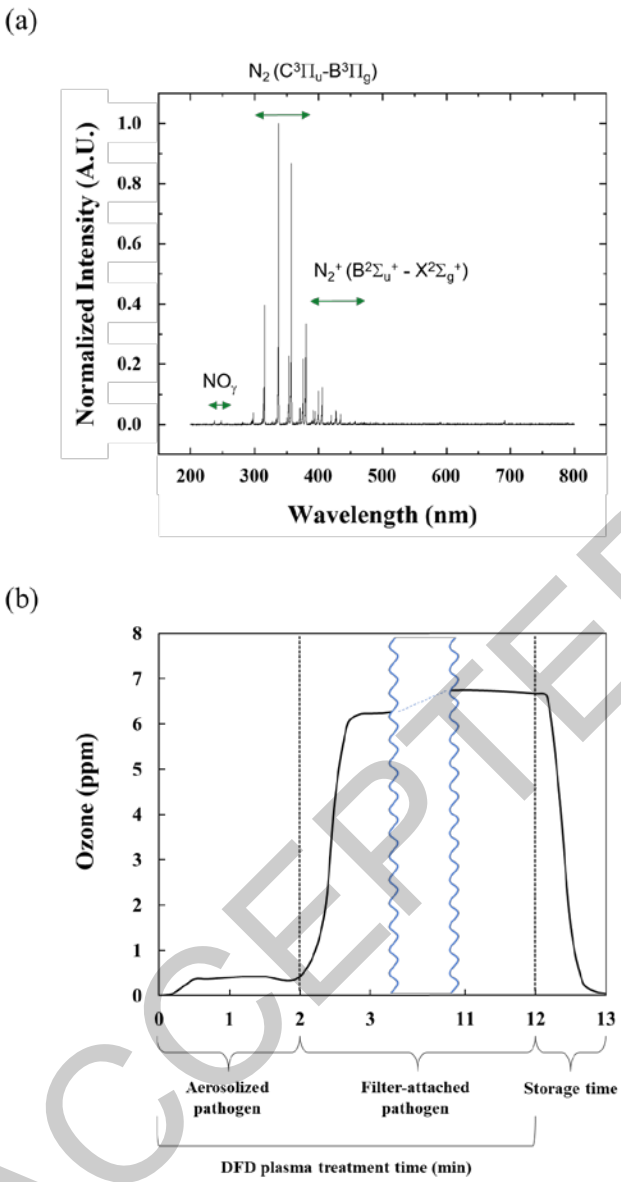


Fig. 3 (a) Optical emission spectrum and (b) ozone concentration during DFD plasma treatment.

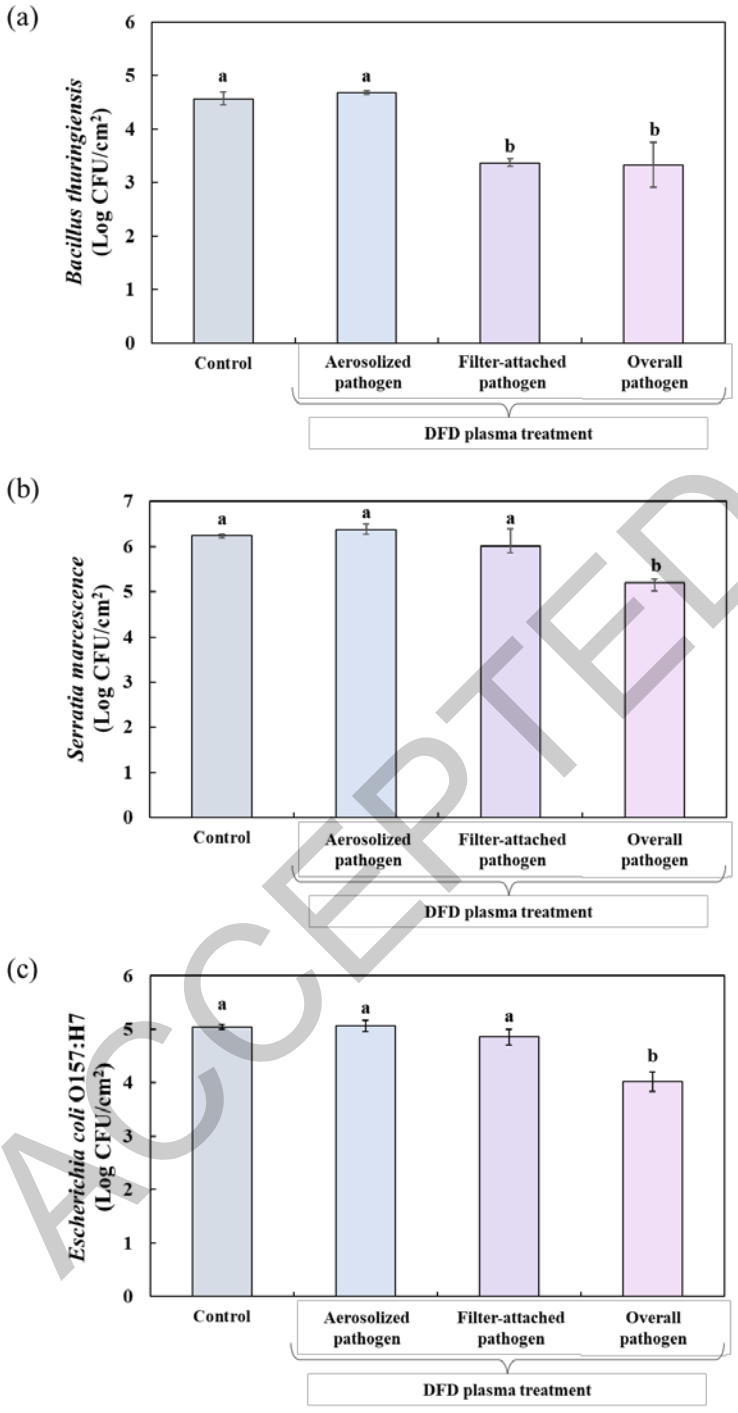


Fig. 4. Experiment I: Inactivation effect of DBD plasma treatment on aerosolized pathogens (2 min), filter-attached pathogens (2 min), and both overall pathogens (aerosolized + filter-attached, each for 2 min). (a) *Bacillus thuringiensis*; (b) *Serratia marcescens*; and (c) *Escherichia coli* O157:H7.

[a,b]The means with different letters are significantly different ($p < 0.05$).

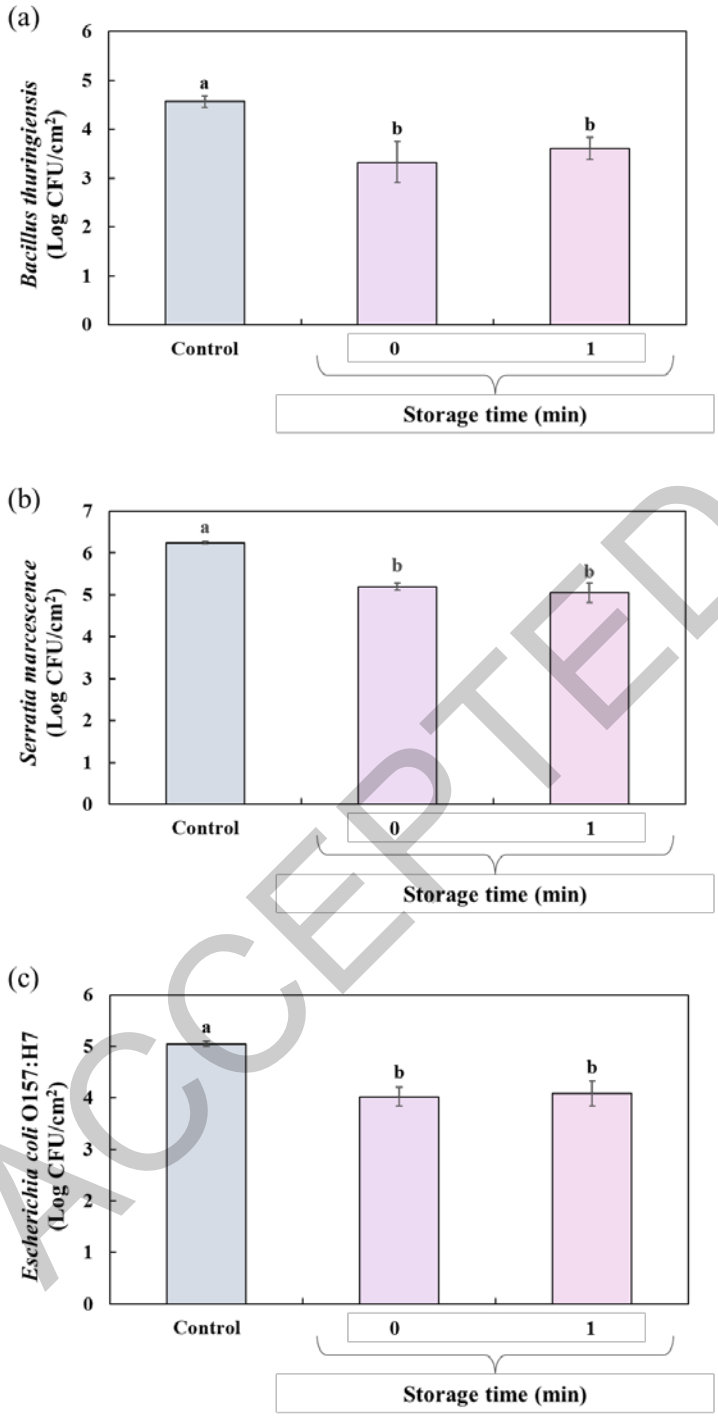


Fig. 5. Experiment II: Inactivation effect on overall pathogens with different storage times (0 and 1 min) after DFD plasma treatment (a) *Bacillus thuringiensis*; (b) *Serratia marcescens*; and (c) *Escherichia coli* O157:H7.

[a,b]The means with different letters are significantly different ($p < 0.05$).

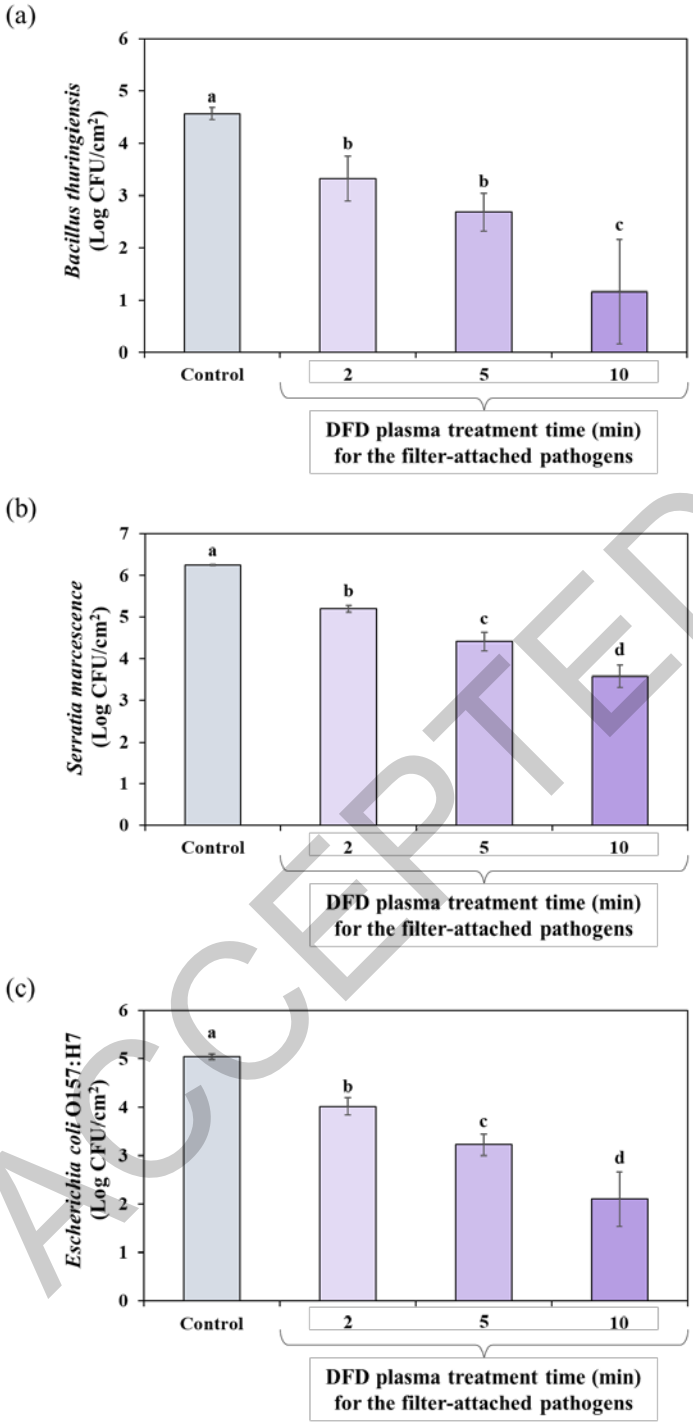


Fig. 6. Experiment III: Inactivation effects of DFD plasma on filter-attached pathogens according to treatment time (2, 5, and 10 min) after continuous treatment of aerosolized pathogens (2 min) (a) *Bacillus thuringiensis*; (b) *Serratia marcescens*; and (c) *Escherichia coli* O157:H7.

[a-d]The means with different letters are significantly different ($p < 0.05$).