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Susceptibility constants of airborne bacteria to dielectric barrier discharge for antibacterial performance evaluation

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HIGHLIGHTS

- ▶ We characterized DBD by measuring concentrations of reactive species and ions.
- ► The inactivation efficiency was deduced using aerosol- and colony-counting methods.
- ► Susceptibility constant (*Z*) was introduced for performance evaluation of DBD.
- ▶ Modified *Z* was suggested for evaluation of DBD reactors of different sizes.
- ▶ Our methodology will be used for design optimization and performance evaluation.

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ABSTRACT

Dielectric barrier discharge (DBD) is a promising method to remove contaminant bioaerosols. The collection efficiency of a DBD reactor is an important factor for determining a reactor's removal efficiency. Without considering collection, simply defining the inactivation efficiency based on colony counting numbers for DBD as on and off may lead to overestimation of the inactivation efficiency of the DBD reactor. One-pass removal tests of bioaerosols were carried out to deduce the inactivation efficiency of the DBD reactor using both aerosol- and colony-counting methods. Our DBD reactor showed good performance for removing test bioaerosols for an applied voltage of 7.5 kV and a residence time of 0.24 s, with η_{CFU} , η_{Number} , and $\eta_{\text{Inactivation}}$ values of 94%, 64%, and 83%, respectively. Additionally, we introduce the susceptibility constant of bioaerosols to DBD as a quantitative parameter for the performance evaluation of a DBD reactor. The modified susceptibility constant, which is the ratio of the susceptibility constant to the volume of the plasma reactor, has been successfully demonstrated for the performance evaluation of different sized DBD reactors under different DBD operating conditions. Our methodology will be used for design optimization, performance evaluation, and prediction of power consumption of DBD for industrial applications.

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1. Introduction

Particles of biological origin, such as bacteria, viruses, fungi, and pollen as well as their fragments that are present in air are referred to as bioaerosols. Bioaerosols can cause serious health hazards when they contaminate a human environment. Diseases from pathogenic bacteria are a major cause of death, accounting for nearly 40% of the total 50 million annual estimated deaths worldwide [1,2]. Severe Acute Respiratory Syndrome (SARS) and the

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threat of avian flu are natural examples illustrating the profound, everyday impacts of bioaerosols on public health [3].

For a long time, researchers have reported that plasma can kill or inhibit the growth of bacteria. Noyce and Hughes [4,5] investigated the bactericidal effect of both negative and positive ions generated by a corona discharge for *Escherichia coli* and *Pseudomonas veronii* bacteria. Tests were conducted in a nitrogen atmosphere to prevent production of ozone (O₃), which is one of the common reactive species generated by an electrical corona in air. After 30 min of exposure to negative and positive ions, the reductions of *E. coli* bacteria were approximately 91% and 98%, respectively [4], and after 60 min of exposure, the reductions of *P. veronii* bacteria were approximately 95% and 86%, respectively [5]. Further, it was suggested that cell death could be due to a change in the outer membrane as a result of ionic interactions.

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Atmospheric pressure plasmas generate many reactive species, and have substantial merit for sterilization system applications [6]. Reactive species such as O, O₃, OH, NO, and NO₂, which are common products of electrical coronas in air, can also sterilize cells. The reactive species can oxidize the cell membrane, protein molecules, and DNA [6,7]. Sari and Fadaee [8] used corona discharge for decontamination of both *Pseudomonas aeruginosa* and *E. coli* bacteria. They reported that adding H_2O_2 makes the method faster and more effective, and suggested that OH radicals play an essential role for such behavior.

Compared to other atmospheric pressure plasmas, such as corona discharge reactors, dielectric barrier discharge (DBD) has the advantage of high efficiency for sterilization in a short period of time [9,10]. Choi et al. [9] and Deng et al. [11] reported that bioaerosols are deactivated by physical or chemical processes in a DBD reactor. The physical process proceeds by positive and negative ions in the discharge streamer, while the chemical process is accomplished by reactive species produced in the DBD reactor. Choi et al. [9] obtained a reduction of 99.99% of E. coli within 70 s. Boudam et al. [12] demonstrated the inactivation of spores of Bacillus subtilis by a DBD, which was operated at atmospheric pressure in a N₂-N₂O mixture. They demonstrated that it is possible to find operating conditions such that spore inactivation with an atmospheric pressure discharge is due to UV radiation and radicals. Fridman et al. [10] reported that Staphylococcus, Streptococcus, and Candida were completely destroyed in less than 15 s when exposed to the

Most previous studies have focused on stationary microorganisms captured on surfaces such as an agar plate, except the works of Liang et al. [13], Gallagher et al. [14], and Vaze et al. [15]. In Liang et al. [13], nonthermal plasma generated by a wireto-plate type DBD reactor was applied to inactivate aerosolized B. subtilis cells and Pseudomonas fluorescence as well as indoor and outdoor bioaerosols. Less than 2% of B. subtilis aerosols survived a plasma treatment of 0.12 s, while none of the P. fluorescens aerosols survived. Exposure of environmental bacterial aerosols to the plasma for 0.06s also resulted in significant inactivation of more than 95% for bacteria. Gallagher et al. [14] demonstrated a 97% reduction in culturable E. coli with a millisecond exposure time in a dielectric barrier grating discharge (DBGD) plasma (one pass through discharge), and a subsequent 99.95% reduction 2 min following treatment. The direct plasma exposure time of 0.73 ms (per pass) allows enough time for bacteria to be attacked by all chemically active components of plasma (charged particles, OH radicals, atomic oxygen, and ozone), which is one explanation for the initial 97% reduction in culturability. Remote exposure to the remaining ozone in the subsequent 2 min direct plasma treatment may account for the additional 99.95% reduction. Vaze et al. [15] suggested that in their DBGD experiment, the main cause of inactivation is the synergetic action of short-lived plasma agents (charges, radicals) and ozone. This synergy creates a toxic environment for the bacteria, ultimately resulting in inactivation.

Bioaerosols are also a kind of airborne particle, and airborne particles can be physically collected by a DBD reactor [16–19]. Some of the bioaerosols are charged by ions generated from the DBD and are deposited (or collected) on electrode surfaces along the electric field. The collection efficiency was reported to increase with the applied voltage and the flow residence time. In this study, we measured the concentration of bioaerosols at the outlet of the DBD reactor both with and without applied power to determine its collection efficiency, which was not reported in the studies of Liang et al. [13], Gallagher et al. [14], and Vaze et al. [15]. The collection efficiency of a DBD reactor is an important factor in determining the performance of DBD reactors. Since a relatively large amount of bioaerosols can be collected in the DBD reactor and only a small portion of the bioaerosols can exit the reactor under high applied

voltage and long flow residence time conditions, simply defining the inactivation efficiency with colony counting numbers for DBD as "on" and "off" may lead to an overestimation of inactivation efficiency of a DBD reactor. In this study, we introduce a methodology to deduce the inactivation efficiency of a DBD reactor using both aerosol- and colony-counting methods.

Since different experimental conditions (such as flow rate, residence time, and power consumption) have been used in previous studies on DBD, direct comparison of inactivation efficiencies of different DBD reactors is not easy. Moreover, for industrial applications, quantitative parameters are needed for design optimization, performance evaluation and prediction of power consumption of DBD systems. As one quantitative parameter, susceptibility has been applied in numerical models to evaluate the antimicrobial effects of an upper-room UVGI (ultraviolet germicidal irradiation) system against bioaerosols [20] and antimicrobial activities of silver and copper nanoparticles against test bacteria [21]. In this study, we introduce for the first time, a susceptibility constant of airborne bacteria to DBD as a quantitative parameter for the performance evaluation of a DBD reactor.

2. Experimental

Staphylococcus epidermidis (ATCC #14990) bacterial cells were obtained from the Korean Culture Center of Microorganisms (Seoul, Korea). *S. epidermidis* is commonly found in a variety of environments, and is a typical airborne microorganism in bioaerosol research [22–26]. *Staphylococci* are common parasites in humans and animals, and occasionally cause serious infections. Although there are many species of *Staphylococcus*, *S. epidermidis* is the most common isolate recovered from clinical specimens [27]. Moreover, *S. epidermidis* is commonly found on the skin or mucous membranes of humans [28], and it is recommended by ISO 14698-1 for testing the biological efficiency of air samplers.

A suspension of *S. epidermidis* was prepared by culturing it overnight, and then inoculating 15 mL of nutrient broth with 0.1 mL of the initial overnight culture followed by incubation for an additional 18 h. The nutrient broth was prepared by dissolving 5 g of peptone and 3 g of meat extract in 1000 mL of sterilized deionized water and then sterilizing the mixture in an autoclave. The tubes containing the suspension were then spun at 6000 rpm for 15 min using a centrifuge (VS-15000N, Vision Scientific Co., Ltd., Korea), and the supernatant was carefully removed. The remaining pellets were then resuspended in sterilized deionized water and vortexed for a few seconds using a vortex mixer (KMC-1300V, Vision Scientific Co., Ltd., Korea). This washing process was repeated three times, after which the pellets were resuspended in sterilized deionized water to obtain a base suspension with a bacterial density of 10⁶ cells/mL.

The DBD reactor was installed in an acryl duct $(5 \text{ cm} \times 5 \text{ cm})$ and consisted of nine parallel plate electrodes that were configured in an alternating fashion, with one electrode being grounded and the next one receiving high AC voltage. The gap spacing between any two electrodes was 5 mm. Each electrode was made from a 0.03-mm thick copper foil (5-mm stream-wise length and 40-mm span-wise length) sandwiched between two 0.25-mm thick dielectric plates (alumina plates, 20-mm stream-wise length and 50-mm span-wise length). Voltage and current were measured using an oscilloscope (WaveRunner 6050A, Lecroy, USA), and their rootmean-square (RMS) amplitude values are indicated in Fig. 1(a). RMS discharge currents were 0.44-1.59 mA for RMS voltages of 0.5–7.5 kV (power consumption: 0.22–11.93 W). The transition to sparking occurred at voltages slightly higher than 8 kV. Fig. 1(b) shows the temporal voltage and current profiles when the RMS voltage and frequency were 7.5 kV and 60 Hz, respectively. The

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