

Application of an atmospheric dielectric barrier discharge for inactivation of bacteria

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Abstract An atmospheric pressure dielectric barrier discharge (DBD) system capable of operating under both positively biased voltage pulses at 500 Hz and sinusoidal AC voltage at 8.5 kHz was constructed. The 500 Hz operation produced DBD plasma that was visually more uniform, whilst optical emission from DBD at 8.5 kHz operation was more intense. Though the inactivation of Gram-positive bacteria, *Bacillus cereus* took longer time than the Gram-negative bacteria, *Escherichia coli* ATCC 25922 and *Salmonella enteritidis*, complete sterilization was generally achieved in about 1 minute of DBD plasma treatment.

Keywords dielectric barrier discharge – inactivation of bacteria – atmospheric pressure discharge

INTRODUCTION

Atmospheric pressure non-thermal plasma has been long implemented in industrial usage with the absence of costly vacuum equipment and system. The non-thermal plasma applications include ozone generation [1], pollution control [2], surface modification [3], lighting sources [4], flat large-area plasma displays [5], and plasma medicine [6]. Several configurations have been proposed to obtain non-thermal plasma at atmospheric pressure such as dielectric barrier discharge, corona discharge, plasma jet, and One Atmosphere Uniform Glow Discharge Plasma (OAUGDP). Dielectric barrier discharge (DBD) is studied in this project as it is easy to construct and its capability of generating low temperature plasma at atmospheric pressure by utilizing the current limiting features of the dielectric barrier is advantageous for application to heat-sensitive biological samples. DBD can be built in such a way that at least one of the electrodes is covered by a dielectric layer and non-equilibrium plasma with electron temperature of 10^4 - 10^5 K with mean energy of range 1 eV can be generated while heavier ions remain low in energy level [6].

Active species such as electrons, ions, metastables, and free radicals generated from DBD are among the reactive agents in its related applications. The effectiveness of the DBD plasma applied to inactivation of Gram-positive and Gram-negative bacteria is examined in this paper.

EXPERIMENTAL SETUP

The DBD system constructed consisted of two parallel copper discs (diameter 3.8 cm, thickness 1 cm) as electrodes. Glass sheet (thickness 2 mm) is used as dielectric covering the high voltage electrode leaving a variable air-gap in-between. Glass is used as dielectric widely in the generation of non-thermal plasma since it is easily available and can be shaped to various arrangements. The DBD device is powered by a home-built high voltage generator (with car-coil transformer) capable of delivering up to 25 kV peak-to-peak at output frequency of 0.1 to 12 kHz as opposed to similar atmospheric pressure glass insulated barrier system which uses lower RF voltage of 1-5 kV for sterilization purpose [7]. Sub-microsecond high voltage pulses of 1-10 kHz were also utilized for the same purpose [8]. Frequency of kHz range is adopted since it's more cost friendly relative to RF system that requires commercial RF generator and impedance matching network.

The high voltage generator constructed is able to produce both (i) positively biased high voltage pulses but at low driving frequencies from 100 Hz to 2.5 kHz, and (ii) sinusoidal AC high voltage at higher driving frequencies of 6 kHz to 12 kHz. The maximum amplitude of the sinusoidal AC DBD voltage depends on the working frequency, f , according to the relation, $f = 1 \div 2\pi\sqrt{LC}$, where L is the inductance of the high voltage generator (6.8 ± 0.2 H) and C is the capacitance of the DBD arrangement (45-70 pF with glass dielectric at varying gap width of 0.5 to 3 mm). Hence, the frequency of operation at fixed applied voltage depends on the DBD arrangement. In the present setup, it is optimized at 8.5 kHz for the sinusoidal voltage and 500 Hz for the positively biased voltage pulses operation.

The dielectric barrier discharge is operated under atmospheric pressure. The discharge voltage is monitored via a Tektronix P6015A H.V. probe, and the current is measured by the Pearson current probe Model 4100. Spectrometer HR4000 from Ocean Optics is employed to measure the optical emission spectra from DBD plasma.

RESULTS AND DISCUSSION

For the bacteria sterilization purpose, the bacteria were exposed to the plasma generated between the electrodes. Two types of configuration were used since

the type of electric field and duration of treatment could have substantial influence to the inactivation effect of bacteria [9]: (i) Positively Biased Voltage Pulses at working frequency of 500 Hz; and (ii) Sinusoidal AC Voltage at working frequency of 8.5 kHz.

Uniformity of the discharge

When the applied high voltage exceeds the breakdown criteria of the air-gap, electrical breakdown is induced and the current filaments created are known as micro-discharges [10]. The intensity and diameter of filaments determine the appearance of the discharge, either diffuse or filamentary.

Breakdown was induced with 13 kV and above for this DBD system. Positively biased voltage pulses driven DBD exhibited more uniform and diffuse discharge; while sinusoidal AC voltage powered discharge was more filamentary in appearance due to memory effect of the dielectric (Fig. 1). The residue charges from previous half breakdown cycle deposited on the dielectric surface facilitated the following half cycle by reinforcing the local field at the identical location. Thus the discharge is always ignited at old location, leading to pattern formation of the micro-discharge over the available surface [10]. The memory effect is more prominent for sinusoidal AC voltage DBD with high operating frequency.

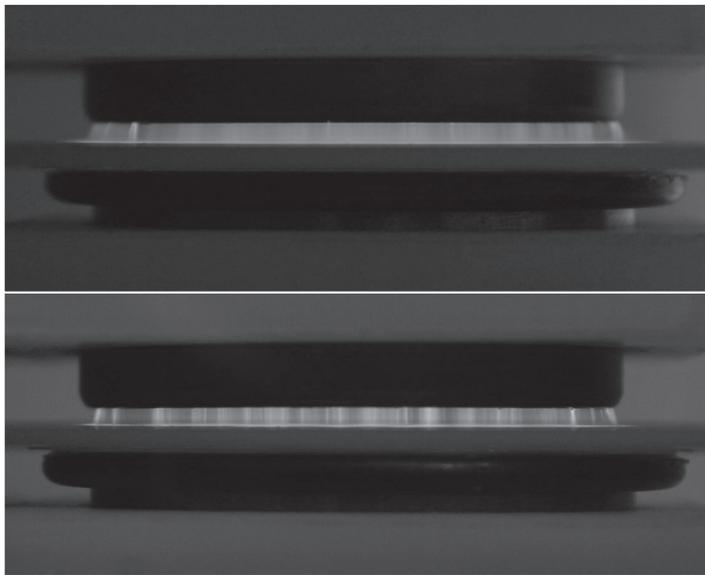


Figure 1. DBD (air-gap width 1.5 mm, single glass dielectric layer) with 500 Hz positive pulses appeared more diffuse (top), and the 8.5 kHz sinusoidal AC voltage powered DBD more filamentary (bottom) [11].

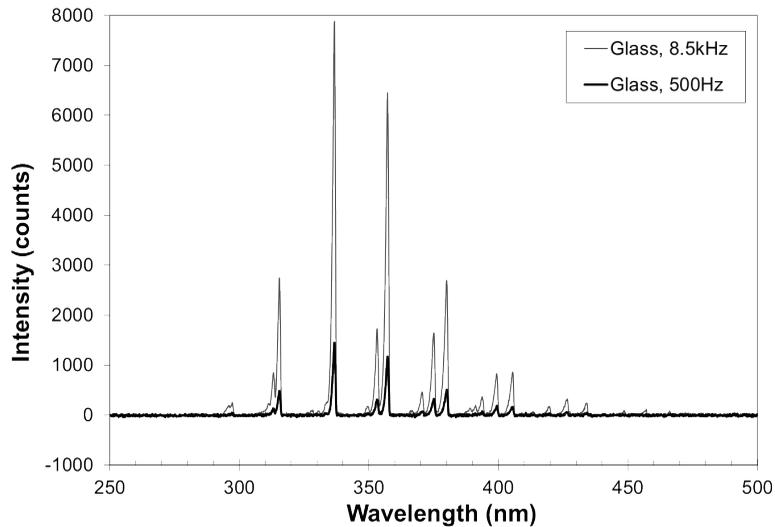


Figure 2. Emission spectra from the DBD plasma. Thinner line (registering higher peak values) represents DBD with sinusoidal voltage excitation, while the thicker line represents DBD excited by positively biased voltage pulses.

Optical emission spectra

The optical emission spectra from the DBD plasma (Fig. 2) consisted mostly of the vibrational and rotational band of the molecular nitrogen second positive system (SPS). This is consistent with the emission from non-thermal plasma in atmospheric air [12].

Inactivation of bacteria

Two types of Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella enteritidis*) and one type of Gram-positive bacteria (*Bacillus cereus*)

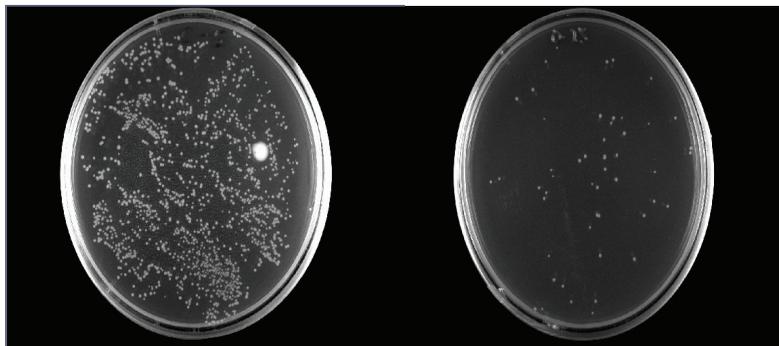


Figure 3. Control bacteria sample (left) and plasma treated bacteria sample with 15s exposure time (right).

were selected to be treated in this project. The survivability of the bacteria was studied with various treatment times (plasma exposure time). Untreated sample was used as control for each set of experiment. The colony forming unit (*cfu*) was determined after 24 hours incubation. The difference in number of *cfu* for the control and treated sample is shown in Figure 3.

The bacteria were exposed to the uniform plasma treatment with positively biased voltage pulsed excitation for the duration of 15, 30, 60, and 120 seconds. For the filamentary plasma via sinusoidal AC voltage excitation, the treatment times were 5, 10, 15, 30 and 60 seconds.

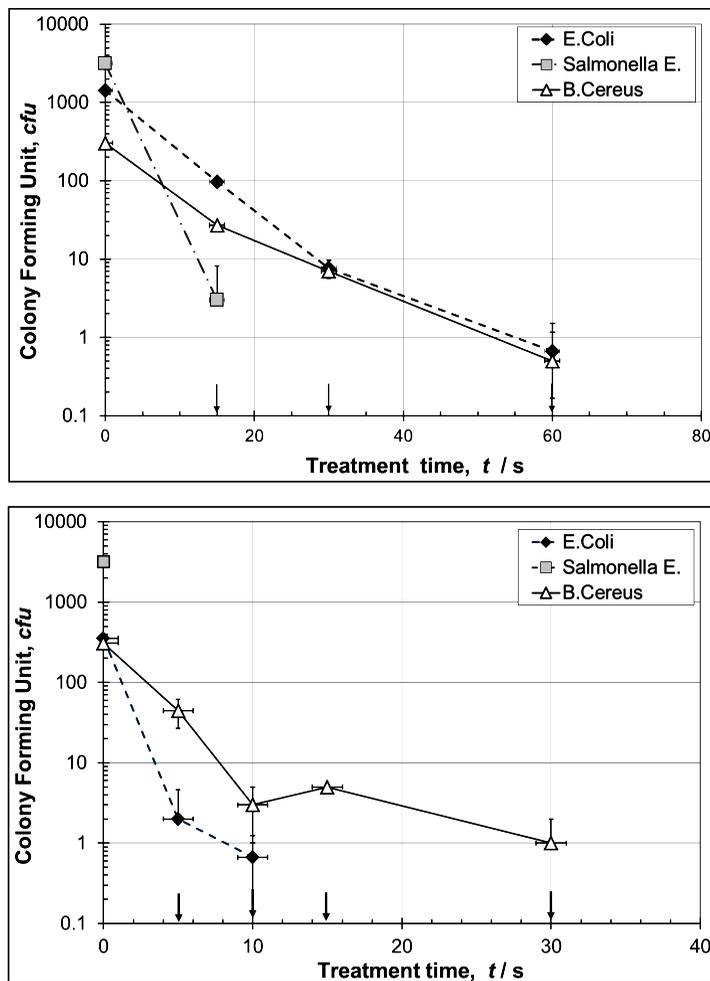


Figure 4. Survival CFU for bacteria with uniform plasma (top) and filamentary plasma (bottom) in logarithmic scale. Arrows at the horizontal axis indicates treatment time used.

The sinusoidal voltage DBD has higher sterilization power in general. Starting with 3170 *cfu* (untreated sample), complete sterilization for *Salmonella enteritidis* was achieved in more than 30 seconds of treatment time for the DBD excited by positively biased pulses (at 500 Hz) while less than 5 seconds was required for sinusoidal voltage DBD at 8.5 kHz. In the case of *Escherichia coli* with initial 1432 and 356 *cfu* in the 500 Hz and 8.5 kHz DBD systems, complete sterilization required more than 1 minute and 10 seconds, respectively. For the *Bacillus cereus* with initial 305-310 *cfu*, more than 1 minute and 30 seconds of treatment was necessary in the respective systems.

Taking into account the higher dilution of Gram-positive bacteria sample (as evident in the reduction by one order of magnitude in the *cfu*), the Gram-positive bacteria *Bacillus cereus* is more resistant to plasma sterilization as compared to the Gram-negative bacteria, *Salmonella enteritidis* and *Escherichia coli*. This is due to the thicker cell wall of Gram-positive bacteria, and may not have undergone significant disruption or morphological change after plasma treatment [13].

CONCLUSION

The plasma generated by the DBD device can effectively disinfect both Gram-negative and Gram-positive bacteria. Though the Gram-positive bacteria take longer time for complete sterilization, complete sterilization for all three samples was achieved in about 1 minute of plasma treatment. The sinusoidal voltage driven DBD system at 8.5 kHz was more efficient in inactivation of the bacteria compared to the positively biased pulsed DBD system at 500 Hz, though the latter produced more uniform plasma.

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