Plasma Medicine, 6(3–4): 447–457 (2016)

Mechanisms of Biocidal Activity of Dielectric Barrier Discharge Air Jet with Misting

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ABSTRACT: Two nearly identical plasma jet–like systems with water misting were investigated for effective pathogen inactivation regimens on *Escherichia coli* in atmospheric conditions. Although the electrode construction is quite similar, we show that the concentration of active species generated at the exit of the discharge is quite different, and inactivation of bacteria by this treatment also varies. Inactivation and production rates of hydrogen peroxide, nitrate, and nitrite in liquid were higher when a single quartz barrier was utilized in the dielectric barrier discharge and one of the electrodes was open stainless steel.

KEY WORDS: plasma, sterilization, disinfection, food safety, plasma agriculture

I. INTRODUCTION

There is a continually growing worldwide demand for safer foods.[1–3] One important concern is the presence of pathogenic organisms on the surface of fresh produce.[2,4] Plasmas, on the other hand, are well-known for their strong antimicrobial properties.[5–8] For plasma medicine, a number of discharges have been developed where plasma can successfully contact living tissue without damaging it and achieve the desired rate of pathogen inactivation—usually within a few seconds of treatment.[9,10] However, delivering plasma treatment to a complex surface of fresh produce can be quite challenging: produce is three-dimensional, frequently multilayered (e.g., a bag of spinach leaves), and the industrial processing rates are very high. For this reason, we have developed a plasma jet–like system where an airstream containing small droplets of water is passed through the discharge and onto the surface of produce. The key challenges, addressed in this paper, are the control of air and water temperature passing through the discharge, and the resulting chemistry generated in the liquid.

II. MATERIALS AND METHODS

A. Preparation of *Escherichia coli* Plates

Trypticase soy agar (TSA) plates (Hardy Diagnostics, CA) were prepared with a solution of *Escherichia coli* O:157 H7 (Rifampicin resistant strain, obtained from Prof. N.

Nitin, University of California at Davis, ATCC #700728). The *E. coli* solution was diluted to 10^4 CFU/mL, and 0.5 mL was spread across each of the TSA plates. Following inoculation, the plates were immediately placed in a 37ºC incubator overnight.

B. Plasma System Setup

For these experiments, we constructed two nearly identical tubular dielectric barrier discharge (DBD) systems. The inner electrode was the same for both systems: quartz-covered solid copper rod; and the outer electrode was a thick stainless steel pipe in one case, and quartz in the second case. In the case of the double-dielectric, significant heating of the outer quartz electrode was observed, and for this reason it was covered with thin sheets of brass with additional forced air cooling to keep the electrode at room temperature. Figure 1 shows the photograph of the two electrodes side-by-side, and Fig. 2 shows the principal schematic of the electrode construction.

Figure 3 shows the schematic of the plasma treatment setup used in these experiments. We used either of the jets positioned 1 inch above the treatment surface, and treated either *E. coli* spread on a Petri dish, or 100 µl drop of water on a concave glass slide. The microsecond-pulsed power supply was identical to the one described elsewhere by the authors.[11] In short, pulses of ~2 µsec width and 28 kV magnitude are delivered to the electrodes at 1,000 Hz pulse repetition rate. Plasma treatment lasted 30 seconds for each testing condition. Droplet nebulization was accomplished using a 2.4 MHz particle generator (241PG, Sonaer Ultrasonics, New York) and these droplets were flown through plasma using either air from a standard air compressor, medical grade dry air from a gas tank, oxygen, or nitrogen. We have compared the treatment with and without droplet nebulization. Humidified air was used to evaluate the effects of water temperature on reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced, mist is produced at room temperature. The mist was produced by placing a heating coil in the container that held the nebulizers, heating the water to boiling.

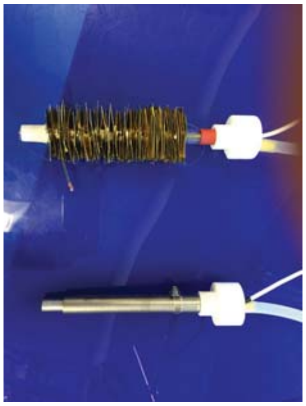

FIG. 1: Photographs of the DBD electrode with quartz (left) and metal (right) outer tubes

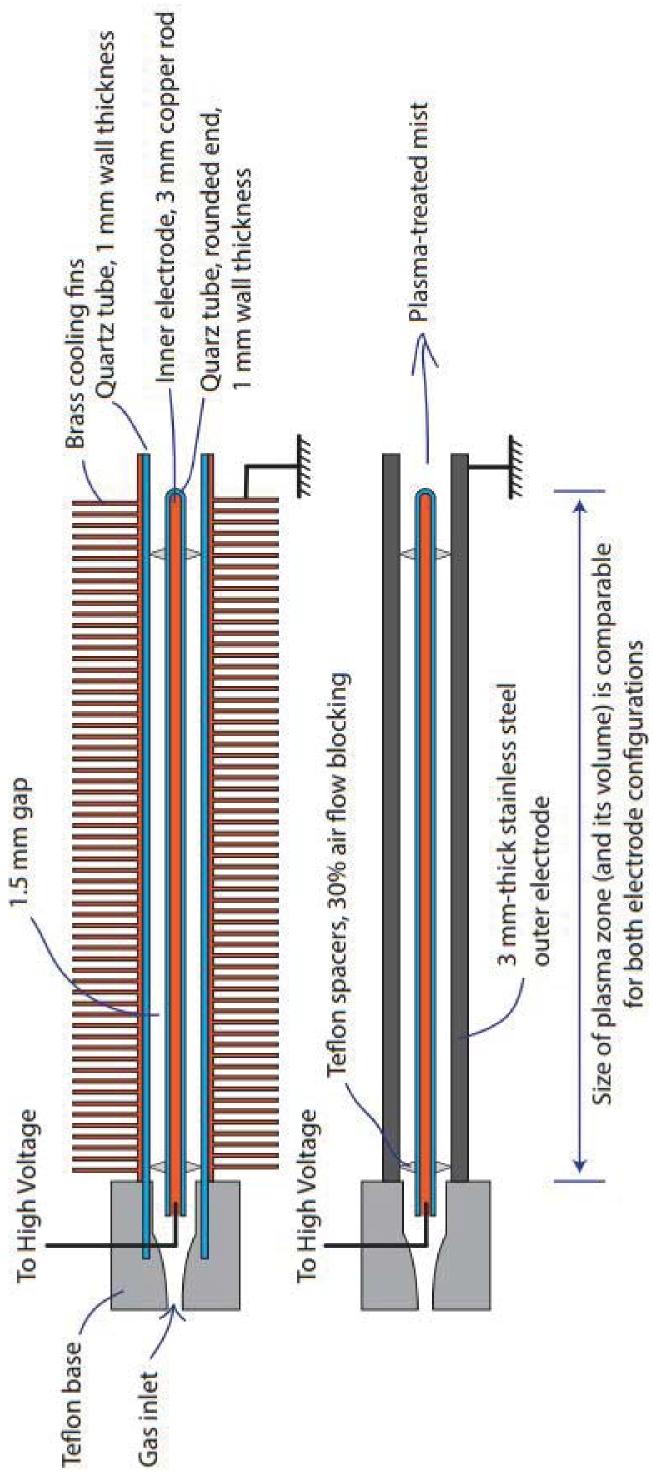

FIG. 2: Schematic of the DBD electrodes

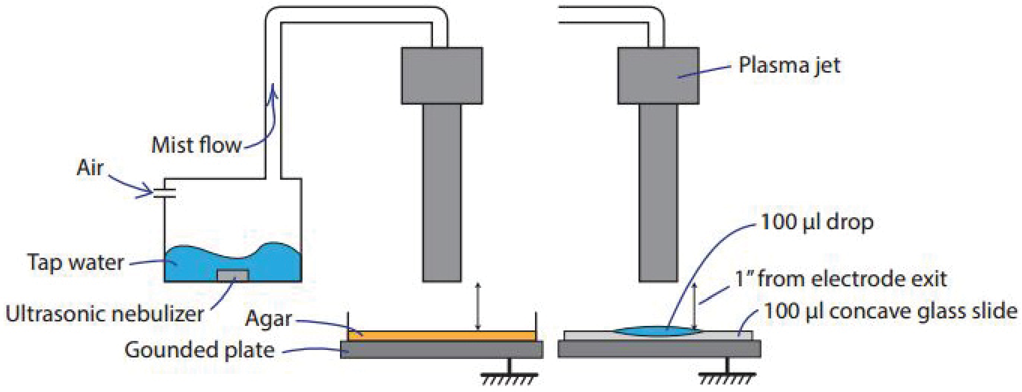

FIG. 3: Schematic of the plasma treatment setup of (left) agar dishes with *E. coli* and (right) 100 µl water drops

Following plasma treatment and subsequent incubation overnight at 37ºC in air, photographs were taken of *E. coli*–covered dishes, and CFUs were counted. pH, H_2O_2, NO_2^-, and NO_3^- indicators (Fisher Scientific) were used for analysis of the water properties.

III. RESULTS

The pH levels of all testing conditions show that, overall, metal tubes resulted in a lower pH than quartz tubes in all testing conditions with the exception of oxygen, with and without mist, as seen in Fig. 4. The lowest pH, pH 2 was achieved by a metal tube with dry air and mist, and humidified air, respectively. Quartz tubes and dry air, with and without mist, and both quartz and metal tubes with oxygen, with and without mist, registered the highest pH of 4. The pH of the metal tubes with compressed air, with and without mist, were constant, in contrast to dry air where the pH increased when mist was introduced.

As seen in Fig. 5, a metal tube with humidified air had the highest levels of hydrogen peroxide at 100 mg/L, whereas a quartz tube with and without mist did not produce any detectable hydrogen peroxide. Metal tubes produced more hydrogen peroxide than quartz tubes in all testing conditions, usually by approximately a factor of two, if not more.

As for nitrite levels, humidified air with a metal tube produced the most at 40 mg/L, as seen in Fig. 6. Quartz tubes and dry air, with and without mist, oxygen gas, and metal tubes with oxygen gas did not produce any detectable NO_2. In both quartz and metal tubes with compressed air with and without mist, 10 mg/L of NO_2 was measured.

Nitrate production followed much the same trend as nitrite production, as far as testing condition that produced them, as seen in Fig. 7. Nitrate production was much greater than that of nitrites, except in the case of quartz tubes with oxygen and mist where it was equal. Compressed air with mist yielded uniform levels of nitrites, but in the case of

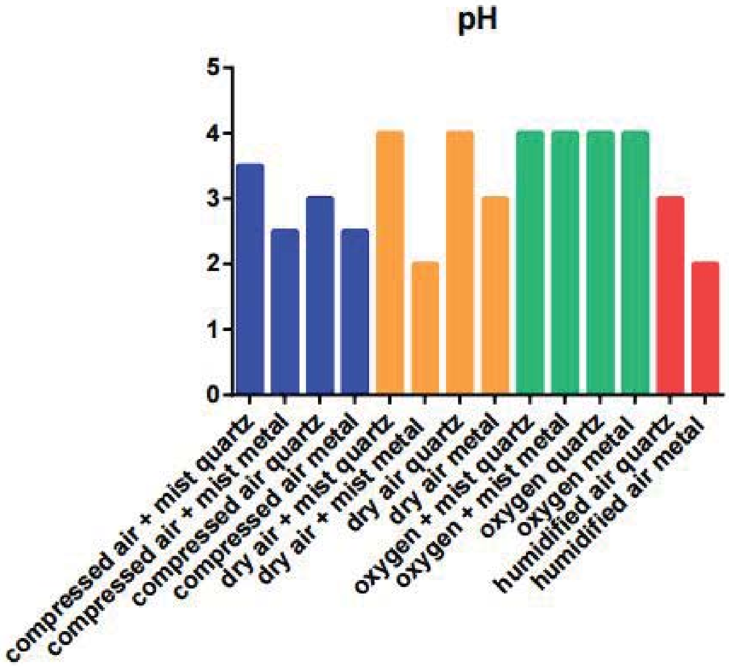

FIG. 4: pH measurement for different plasma conditions

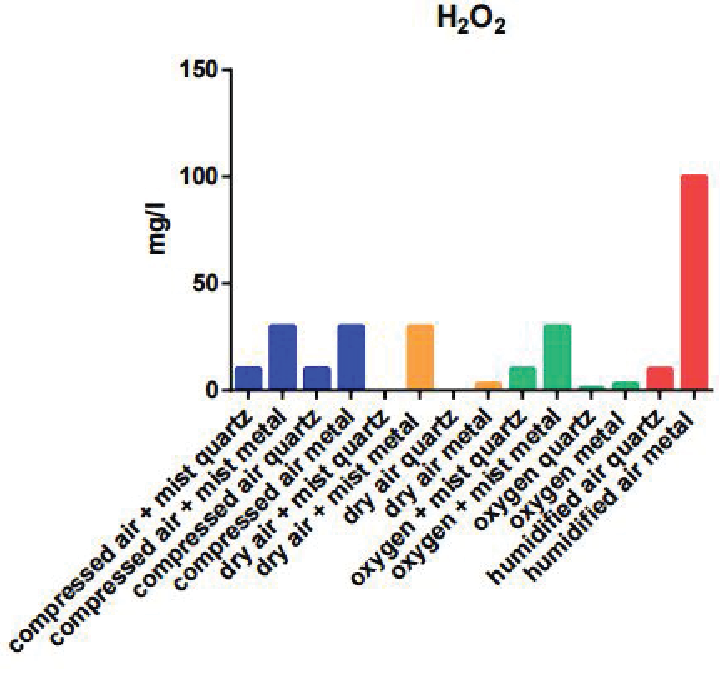

FIG. 5: Levels of hydrogen peroxide for each testing condition (mg/L)

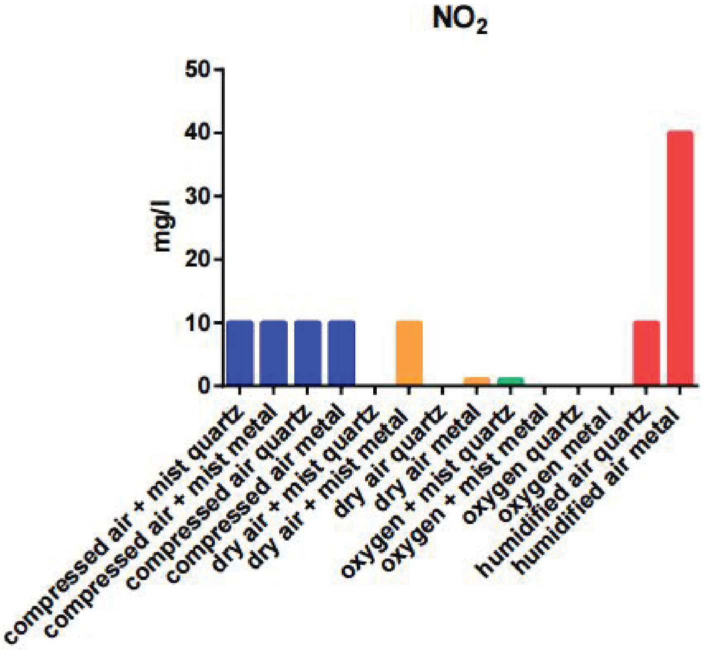

FIG. 6: Nitrite levels for each testing condition (mg/L)

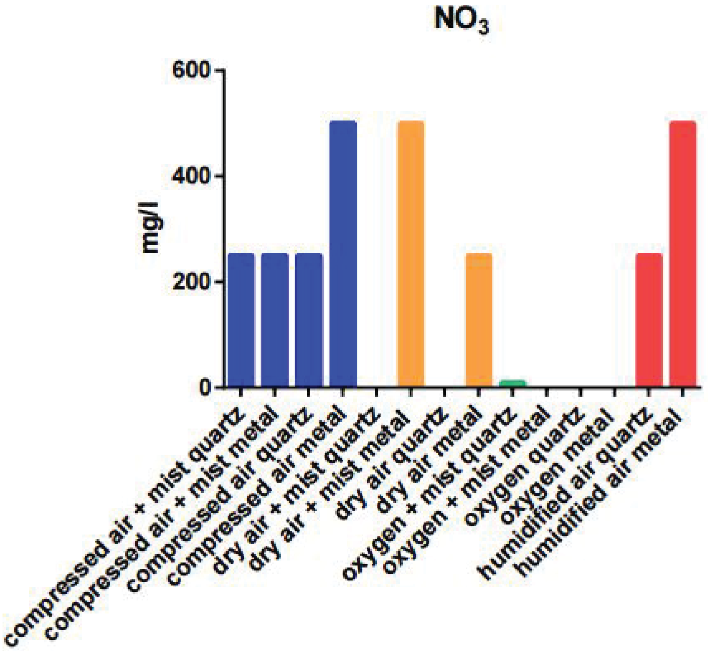

FIG. 7: Nitrate levels for each testing condition (mg/L)

nitrates, the metal tube with compressed air produced two times more nitrates than any other compressed air testing condition.

Differences between humidified air, dry air, and compressed air, both with mist, were seen for all four testing criteria, as well in TSA plate treatment; however, they are essentially the same treatment, with humidified air having a higher temperature. This shows that temperature does have an effect on reactive species produced and on biocidal activity.

The results of the plasma treatment of *E. coli* plates after 24-hour incubation are shown in Fig. 8. When analyzing the treated plates, it is clear that metal tubes perform better than quartz in killing bacteria, because larger areas on the plates are cleared of *E. coli*. Metal tubes and oxygen, with (Fig. 8 X, Z) and without mist (Fig. 8 T, V) performed the best out of all testing conditions, leaving an average of four colonies on the plate. Quartz tubes with dry air and mist (Fig. 8 O, Q) performed worst of all testing conditions and look almost indistinguishable from the untreated control plates (Fig. 8 A, B). In the case of compressed air and dry air, more bacterial colonies were killed without mist (Fig. 8 C–F, K–N) than with mist (Fig. 8 G–J, O–R) for both quartz and metal tubes. *E. coli* plates treated with metal tubes and oxygen showed no changes in result, regardless of whether mist was combined with oxygen. However, the same cannot be said for quartz tubes. Humidified air (Fig. 8 AA–AD) was also more effective than compressed air with mist for both metal and quartz tubes (Fig. 8 G–J).

From the combination of ROS and RNS detection testing, and plasma treatment for biocidal activity, it can be deduced that the differences between the metal and quartz tubes result from RNS. The differences may not be detected because of inexact measurements from the indicator strips, or because the RNS is one that has not been tested for.

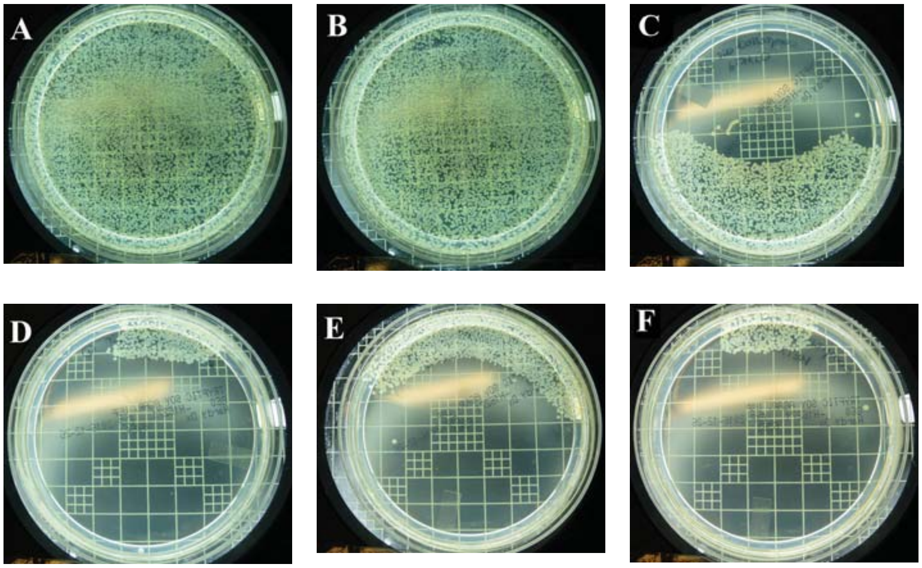

FIG. 8.

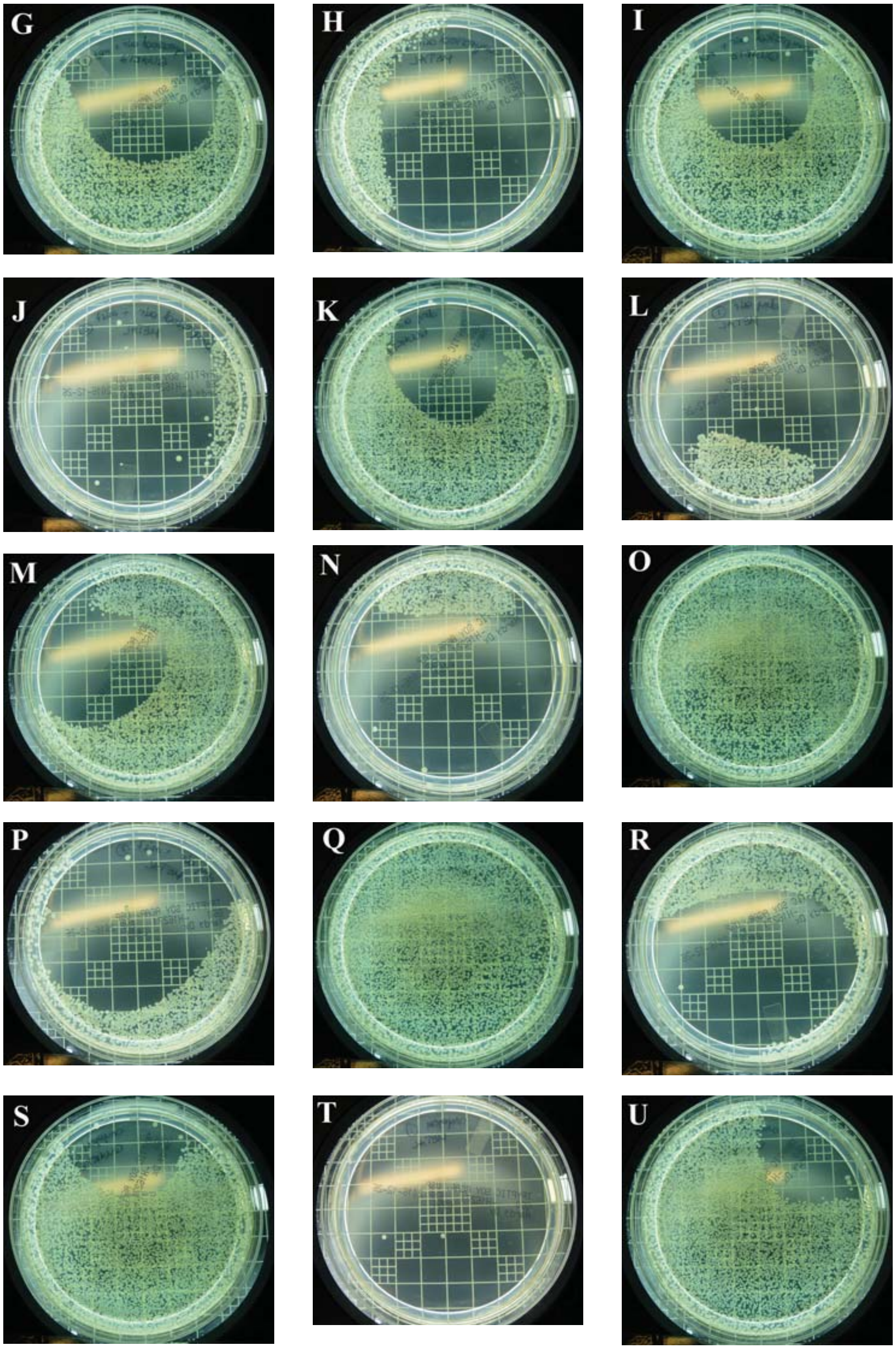

FIG. 8.

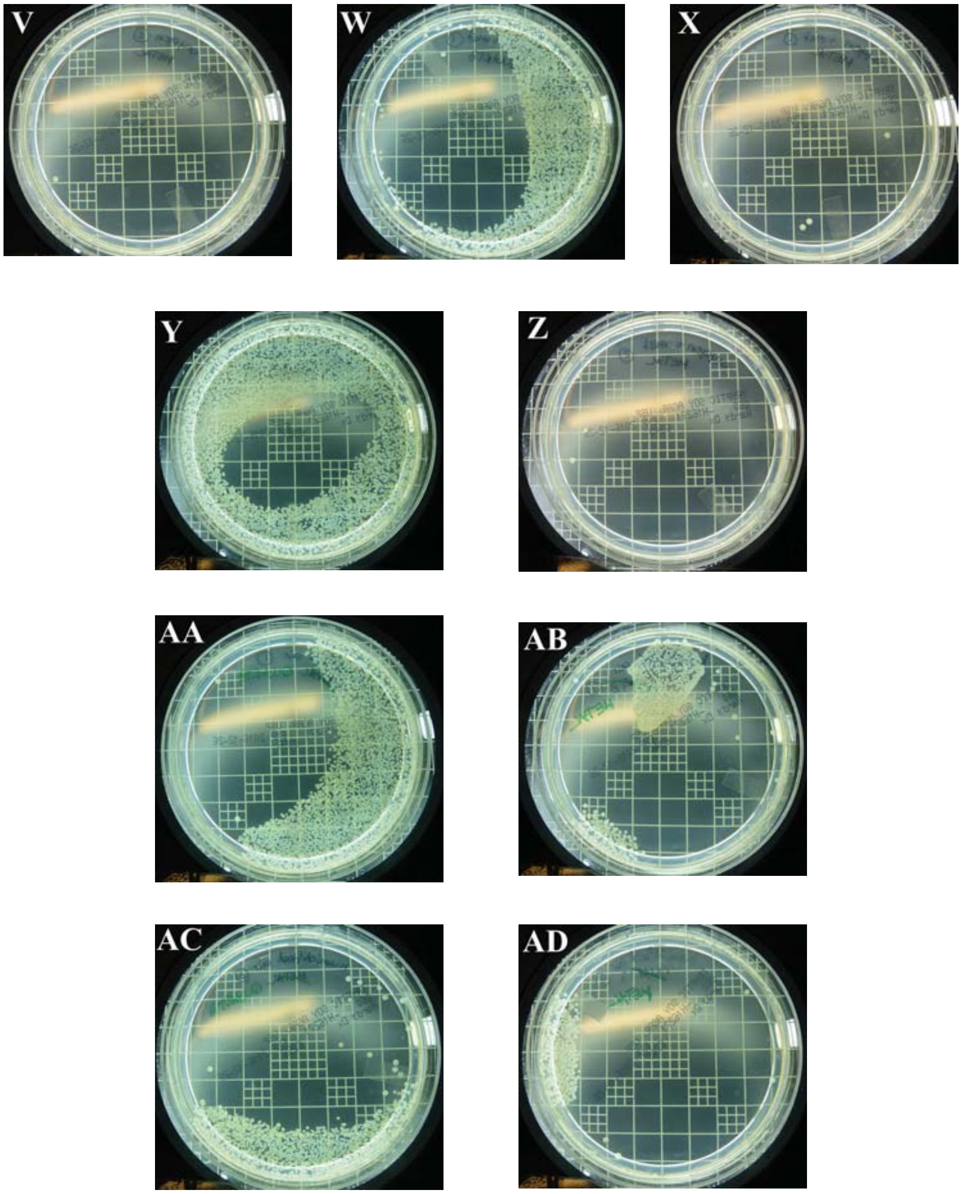

FIG. 8: Inoculated TSA plates 24 hours after plasma treatment under each testing condition. Controls (A and B); quartz tube and compressed air (C and E); metal tube and compressed air (D and F); quartz tube and compressed air with mist (G and I); metal tube and compressed air with mist (H and J); quartz tube and dry air (K and M); metal tube and dry air (L and N); quartz tube and dry air with mist (O and Q); metal tube and dry air with mist (P and R); quartz tube with oxygen (S and U); metal tube with oxygen (T and V); quartz tube and oxygen with mist (W and Y); metal tube and oxygen with mist (X and Z); quartz tube with humidified air (AA and AC); and metal tube with humidified air (AB and AD)

IV. DISCUSSION

We have investigated two very similar plasma jet–like systems: one with a double quartz barrier, quartz DBD or “qDBD,” and one with single quartz barrier, metal DBD or “mDBD.” Both qDBD and mDBD were tested in the amount of reactive species they generate in liquid, under different discharge conditions, and the *E. coli* inactivation rate on agar surface. Both systems show very similar visual behavior: the same surface temperature of the electrodes (qDBD utilizes forced air cooling to keep electrodes at room temperature), visually similar plasmas, and visually the same amount of plasma-treated mist they generate. However, mDBD consistently showed lower pH and higher concentration of H_2O_2, NO_2^-, and NO_3^-, generated in the liquid. The observed effects on *E. coli* inactivation showed the same trend with significantly better inactivation shown from the treatment with mDBD in all of the tested conditions.

V. CONCLUSION

The presented results show a significant difference in the biological effects from two plasma discharges that are nearly identical, with the exception of a single quartz barrier used in one and two barriers in the other. Deeper understanding of plasma-chemical mechanisms of reactive species generation in these discharges is clearly lacking, and the authors plan to continue these investigations, because this is pertinent in developing plasma systems for food safety and fresh produce disinfection.

ACKNOWLEDGMENT

This research was supported, in part, by funding from USDA-NIFA Program on Enhancing Food Safety through Improved Processing Technologies (A4131) grant number 2015-68003-23411.

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