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Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure

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Abstract

Recently, non-equilibrium, atmospheric pressure air plasmas have been shown to possess excellent germicidal properties. A number of studies have shown that air plasmas are capable of inactivating a wide range of microorganisms in the matter of few seconds to few minutes. However, until now little information regarding quantitative measurements of the various plasma agents that can potentially participate in the inactivation process has been published. In this paper, emission spectroscopy and gas detection are used to evaluate important plasma inactivation factors such as UV radiation and reactive species. Our measurements show that for non-equilibrium, atmospheric pressure air plasmas, it is the oxygen-based and nitrogen-based reactive species that play the most important role in the inactivation process.

Keywords: Microorganism; Air plasma; Discharge; Reactive species; Sterilization

1. Inroduction

The inactivation of harmful microorganisms such as bacteria can be achieved by chemical and/or physical means, such as heat, chemical solutions and gases, and radiation [1]. Most conventional sterilization techniques are associated with some level of damage to the material or medium supporting the microorganisms. This does not present a problem in cases where material preservation is not an issue. However, in cases where it is imperative not to damage the materials to be sterilized, conventional methods are either not suitable at all or offer very impractical and/or tedious and time consuming solutions. This situation led to the development of new techniques that are at least as effective as established ones, but with added superior characteristics such as short processing times, non-toxicity, and medium preservation. Amongst these new methods, non-equilibrium atmospheric pressure plasmas have been shown to present a great promise [2–5].

In this paper, the identification and potential role of each inactivation agent generated by the plasma is assessed. Generally, various gas mixtures can be used to optimize the production of an agent or another and to optimize the efficiency of the inactivation process. The analysis presented here, however, is for low temperature atmospheric pressure plasmas generated in air. For information on plasma sterilization using other gas mixtures such as O_2/CF_4, or at low pressures, the reader is referred to Refs. [6–8].

2. Identification of the inactivation factors and assessment of their roles

Under plasma exposure, bacterial cells can be inactivated by one of four known factors or by a synergistic combination of these. These factors are the heat, UV radiation, charged particles, and reactive neutral species. The extent of the influence of each factor depends on the plasma operating parameters such as power and gas mixture and flow rate. Here, we present relative, and when possible, absolute measurements of the presence of these agents in an atmospheric pressure air plasma generated by a Dielectric Barrier Discharge (DBD). Since our experiments are conducted with the biological sample placed at some distance from the plasma (remote exposure), the effects of charged particles (electrons and ions) will not be discussed. A comprehensive study of the effects of charging bacterial cells by a plasma can be found in Ref. [9].

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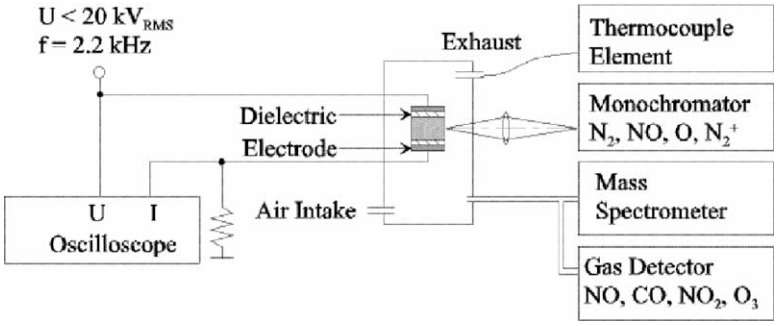

Fig. 1. Experimental setup of DBD air plasma generator and related diagnostics.

A schematic of the experimental setup showing the DBD and the diagnostics used in our evaluations is presented in Fig. 1. The electrodes of our DBD consist of two symmetrical aluminum plates covered by 1 mm thick sheets of alumina (Al_2O_3). The distance between the electrodes is adjustable, up to 1.25 cm. Water cooling of the electrodes allows us to keep the temperature close to room temperature. The applied voltage and the discharge current are monitored by means of a high voltage probe and a current viewing resistor, respectively. The discharge was operated at power levels up to 20 W at an electrode separation of about 7 mm. Optical emission spectroscopy, mass spectroscopy, and gas detection (for NO_2, NO, and O_3) were used to diagnose the plasma contents.

2.1. Heat and its potential effect

It has long been known that heat has detrimental effects on living cells. Therefore, heat-based sterilization techniques were developed and commercially used for applications that do not require medium preservation. In heat-based conventional sterilization methods, both moist heat and dry heat are used. In the case of moist heat, such as in an autoclave, a temperature of 121 °C at a pressure of 15 psi is used [10]. Dry heat sterilization requires temperatures close to 170 °C and treatment times of about 1 h [10].

To assess if heat plays a role in the case of an air plasma, the gas temperature in the discharge was determined by comparing the experimentally measured rotational bands structure of the 0–0 transition of the 2nd positive system of nitrogen with simulated spectra at different temperatures. In addition, the temperature in a sample, placed 2 cm away from the discharge, was measured by a thermocouple probe.

Fig. 2 shows the measured and calculated rotational bands of the 0–0 transition of the 2nd positive system of N_2, for a power of 10 W. It indicates that the gas temperature remains close to room temperature. A variation in power from 2 to 15 W showed no significant change in the relative spectral distribution. This indicates a power-independent temperature in the range between 2 and 15 W at a gas flow rate of 10 l/min. The gas temperature for various gas flow rates at a power consumption of 10 W was also investigated. The results are shown in Fig. 3. For a very low flow (0.5 l/min), a gas temperature of 340 K was found. Increasing the airflow causes the gas temperature to approach room temperature (300 K).

Fig. 4 shows the increase in the temperature of the biological sample under treatment for various dissipated power

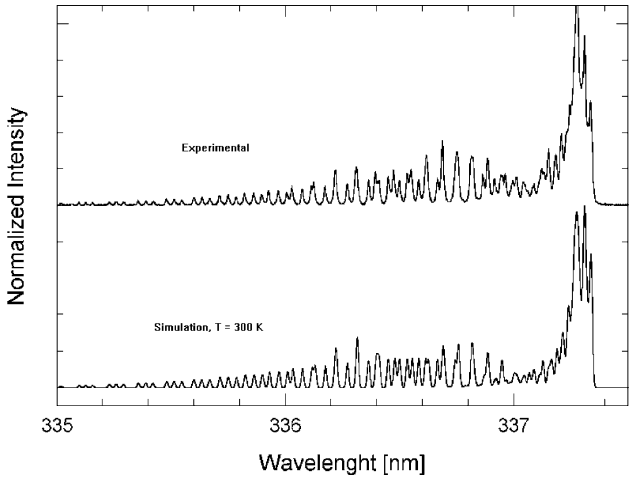

Fig. 2. Measured and calculated rotational bands of the 0–0 transition of the second positive system of nitrogen. The spectra are intentionally shifted vertically for better comparison.

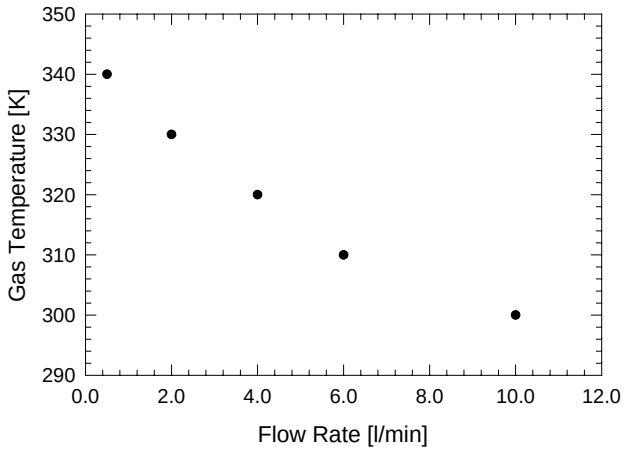

Fig. 3. Gas temperature vs. gas flow rate for a power of 10 W.

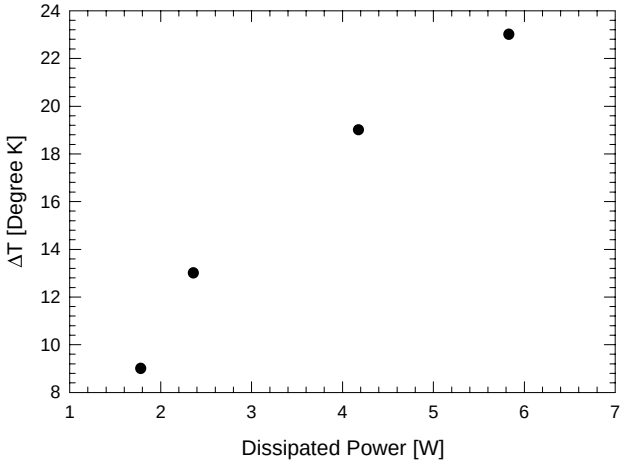

Fig. 4. Increase of sample temperature vs. plasma dissipated power.

levels, as measured by a thermocouple. At our typical running power levels, a maximum increase of 21° was observed. Therefore, based on these measurements no substantial thermal effects on bacterial cells are expected.

2.2. Ultraviolet radiation and its potential role

From early times humans have known that sunlight has beneficial hygienic effects. This is of course due the presence of UV radiation in the sunlight spectrum. Amongst UV effects on cells of bacteria is the dimerization of thymine bases in their DNA strands. This inhibits the bacteria's ability to replicate properly. Wavelengths in the 220–280 nm range and doses of several mW s/cm^2 are known to have the optimum effect [11].

Spectroscopic and absolute power measurements were conducted to quantify the UV contribution to the inactivation process in the case of an air plasma. Our results show that no significant UV emission occurs below 285 nm. This is illustrated in Fig. 5. Power measurements with a calibrated UV detector in the 200–300 nm wavelength region revealed that the power density of the emitted UV radiation is below 50 μW/cm^2 and is essentially independent of the air flow rate. At this power levels we expect the UV not to play a significant direct role in the sterilization process by low temperature air plasmas.

2.3. Reactive species and their role

In high-pressure non-equilibrium plasma discharges, reactive species are generated through various collisional pathways, such as electron impact excitation and dissociation. Reactive species play an important role in all plasma–surface interactions. Air plasmas are excellent sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as atomic oxygen (O), ozone (O_3), hydroxyl (OH), NO, NO_2, etc. Some reaction pathways that lead to the generation of these species in air plasmas are:

$$e + O_2 \rightarrow e + O_2(A^3 \sum_u^+) \rightarrow e + O(^3P) + O(^3P)$$

$$e + O_2 \rightarrow e + O_2(B^3 \sum_u^-) \rightarrow e + O(^1D) + O(^3P)$$

$$O + O_2 + M \rightarrow O_3 + M$$

$$N + O + N_2 \rightarrow NO + N_2$$

$$NO + O_3 \leftrightarrow NO_2 + O_2$$

$$NO_2 + O_2 + hv \rightarrow O_3 + NO$$

$$H_2O + O_3 \leftrightarrow O_2 + 2OH$$

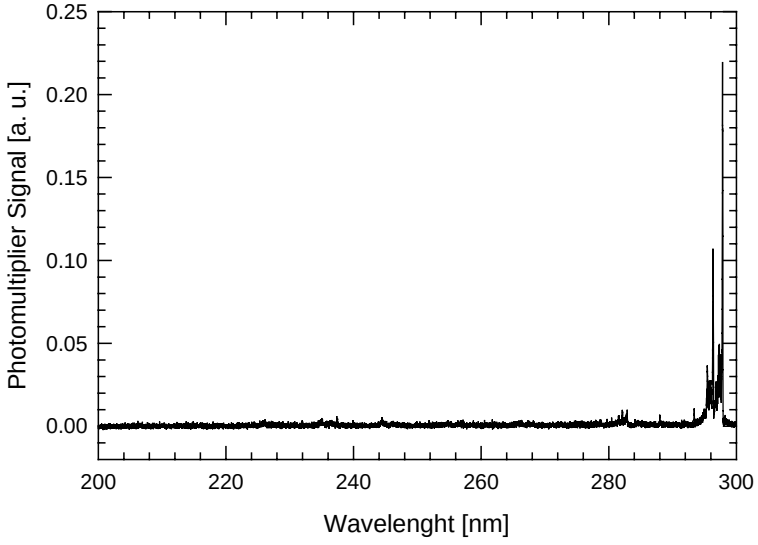

Fig. 5. UV spectrum of a DBD in air in the 200–300 nm wavelength range.

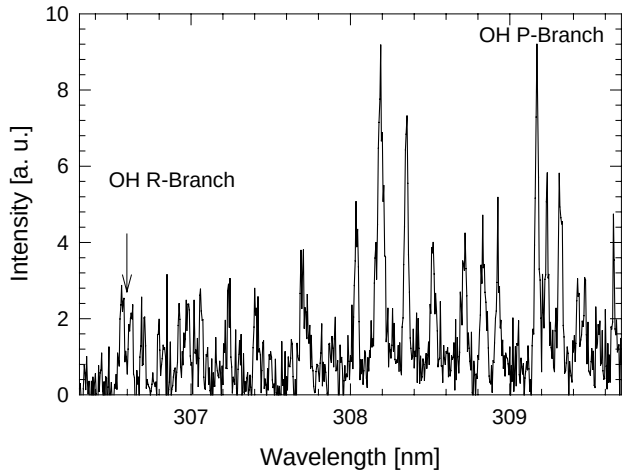

Fig. 6. Emission spectrum from a DBD in air showing OH band heads.

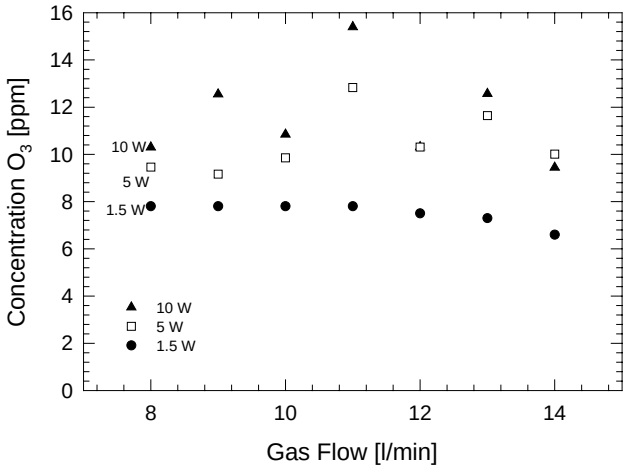

Fig. 8. Ozone concentration generated in a DBD in air as a function of air flow rate and at three power levels (1.5, 5, and 10 W).

$$e + H_2O \rightarrow OH + H + e$$

$$O + H_2O \rightarrow 2OH$$

The following are measurements of oxygen, hydroxyl, ozone, and nitrogen dioxide obtained from a DBD operated in atmospheric pressure air. Relative concentration of atomic oxygen in the DBD, as measured by detecting the oxygen lines at 615.597 and 615.678 nm, showed that the concentration of atomic oxygen decreased less than 20% as the flow rate was increased from 1 to 18 l/min. The presence of OH was measured by means of emission spectroscopy, looking for the rotational band of OH A–X (0–0) transition. This molecular band has a branch at about 306.6 nm (R branch) and another one at 309.2 nm (P branch). Fig. 6 shows the emission spectrum in the range between 306 and 310 nm and it indicates the OH band heads. Fig. 7 shows the relative concentration of OH in the discharge as a function of the air flow rate and dissipated power, assuming that the rotational band intensity represents the OH concentration. The ozone concentration was measured for varying flow rates and at various power levels by a calibrated ozone detector. The results are shown in Fig. 8. Ozone germicidal effects are caused by its interference with cellular respiration. Nitrogen dioxide was measured as a function of the air flow rate and for different power levels by a calibrated gas detecting system and the results are shown in Fig. 9.

The reactive species mentioned above have direct impact on the cells of microorganisms, and especially on their outermost membranes. These membranes are made of lipid bilayers, an important component of which is unsaturated fatty acids. The unsaturated fatty acids give the membrane a gel-like nature. This allows the transport of the biochemical

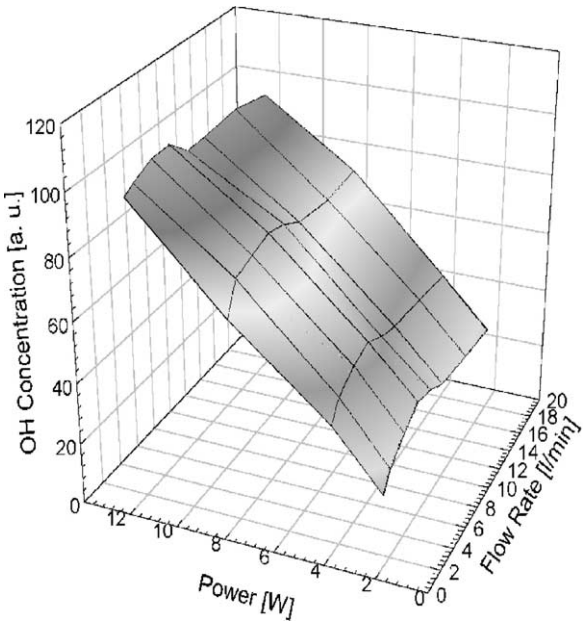

Fig. 7. Relative OH concentration as a function of plasma dissipated power and air flow rate.

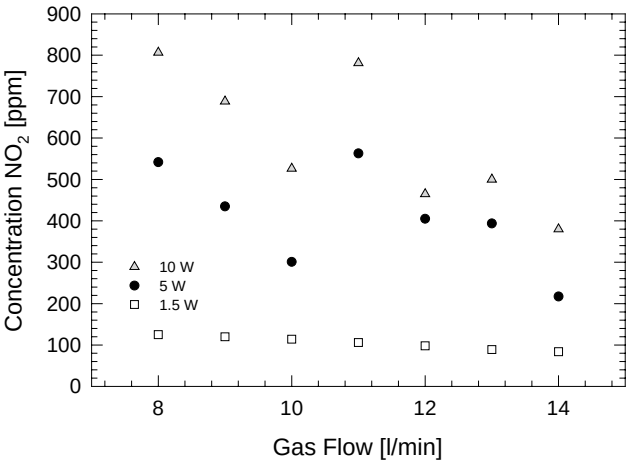

Fig. 9. Concentration of nitrogen dioxide generated in a DBD in air as a function of air flow rate and at three power levels (1.5, 5, and 10 W).

by-products across the membrane. Since unsaturated fatty acids are susceptible to attacks by hydroxyl radical (OH) [12], the presence of this radical can therefore compromise the function of the membrane lipids whose role is to act as a barrier against the transport of ions and polar compounds in and out of the cells [13]. Imbedded in the lipid bilayer are protein molecules which also control the passage of various compounds. Proteins are basically linear chains of aminoacids. Aminoacids are also susceptible to oxidation when placed in the radical-rich environment of the plasma. Therefore, the reactive species generated by air plasmas are expected to greatly compromise the integrity of the cells of microorganisms, leading to their eventual destruction.

3. Correlation between the presence of reactive species and inactivation kinetics

One kinetics measurement parameter, which has been used extensively by researchers studying sterilization by plasma, is what is referred to as the "*D*" value (Decimal value). The *D*-value is the time required to reduce an original concentration of microorganisms by 90%, or if the "kill' curve is plotted on a semi-logarithmic scale, the *D*-value is determined as the time for a one $\log_{10}$ reduction.

To show the effects of reactive species on the destruction of bacteria, kill curves were plotted for three different gaseous conditions: helium only, 97% helium/3% oxygen mixture, and air, all at atmospheric pressure. Spores of the *Bacillus* genus were used since they are hard to kill and are accepted metrics for biological sterilization. When helium is used, only very small concentrations of radicals originating from impurities are expected. When helium is mixed with oxygen, oxygen-based species such as O and O_3 are generated. When air is used, both oxygen-based and nitrogen-based species are generated.

Fig. 10 shows a comparison between the inactivation kinetics in the case of helium and when a 97%–3% helium/oxygen mixture, respectively, was used. After 10 min of treatment time the surviving spore population percentage

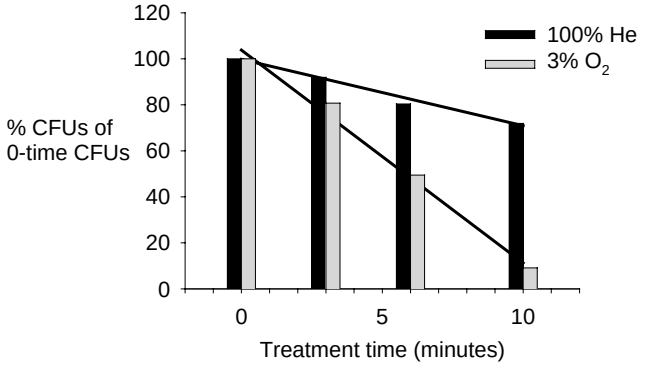

Fig. 10. Percent of surviving *Bacillus* spores vs. plasma treatment time for helium (black) and helium/oxygen mixture (97% He, 3% O_2) (gray).

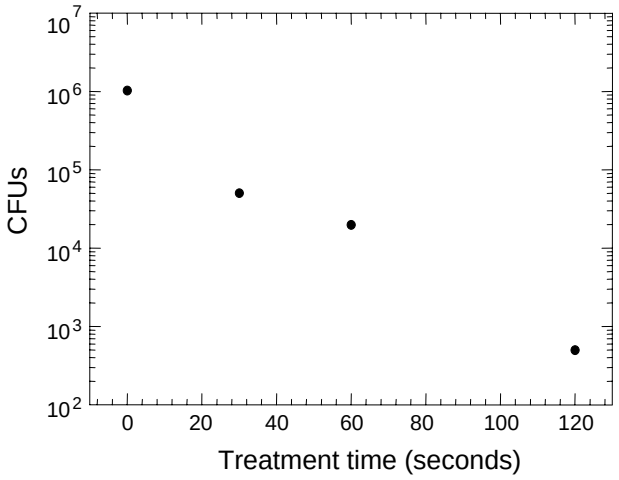

Fig. 11. Colony forming units of *Bacillus* spores vs. treatment time by a low temperature, atmospheric pressure air plasma.

was still much greater than 10%, when only helium was used as the operating gas. In fact the *D*-value in this case was greater than 20 min. When the helium/oxygen mixture was used, as shown in Fig. 10, a *D*-value of 10 min was achieved. Fig. 11 shows the inactivation kinetics of an air plasma. A *D*-value close to 20 s was achieved in this case. This is a 30 times faster inactivation process than the previous case. Since heat and UV radiation were shown not to play an important role for cold air plasmas, the dramatic increase in inactivation efficacy is attributed to the presence of the chemically reactive species such as NO, NO_2, O, O_3, etc. . . . .

4. Conclusion

Low temperature, atmospheric pressure plasmas have been shown to possess very effective germicidal characteristics. Their relatively simple and inexpensive designs, as well as their non-toxic nature, give them the potential to replace conventional sterilization methods in the near future. This is a most welcome technology in the healthcare arena where re-usable, heat sensitive medical tools are becoming more and more prevalent.

In this paper, based mainly on non-intrusive optical diagnostics and gas detection systems, we conclude that in the case of low temperature air plasmas, it is the highly reactive species such as O, OH, and NO_2 that play the most crucial role in the destruction of microorganisms. Heat and UV radiation may play a secondary role, but we expect their effects to be either minimal or indirect.

Acknowledgements

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