JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 10, No. 8, August 2008, p. 2077 - 2081

Optimum working parameters for the plasma needle used for bacterial deactivation

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This paper presents the operating stability diagram for atmospheric pressure non-thermal He plasma with the aim of establishing the optimum working parameters for bacterial deactivation applications. The diagram indicates that there are four regions and three different shapes for the plasma. The plasma temperature was determined via thermocouple method. The atomic and molecular species present in the plasma were identified by emission spectroscopy technique. The excitation temperature for He is around 0.2-0.25 eV. *Escherichia coli* cultures were deactivated successfully, results function of treatment time or gas flow-rate are presented and are compared with other sterilization techniques.

(Received March 1, 2008; accepted June 30, 2008)

Keywords: Non-thermal plasma, Stability diagram, Bacterial deactivation

1. Introduction

The plasma needle is a device which facilitates the ignition and the sustainment of atmospheric pressure non-thermal plasma with very promising biomedical applications [1-5]. It could be sustained in He gas flow (Ar, N_2, He and air mixtures may be used too), around a sharp metallic electrode, using RF electromagnetic fields with frequencies around 13.56 MHz, being a source of charged particles, UV radiation and active species and it has been studied extensively in many laboratories from both experimental [6-10] and theoretical point of views [11-13].

Such plasma was obtained successfully in our laboratory, at atmospheric pressure in He flow, below 1 MHz and around 10 MHz, with RF power levels up to 3 W, with a plasma needle type device [14, 15]. As compared to the experimental set-up used by other authors to generate the plasma needle, we have exploited the possibility of obtaining various RF electromagnetic fields from the resonant circuit of the free running type RF oscillator, necessary to generate discharges at atmospheric pressure, the plasma being intrinsic part of the resonant circuit. In our set-up, matching conditions could be achieved easily by adjusting the coupling factor between the oscillating circuit's coil and the reaction coil, thus no external matching network is needed [16].

The aim of this paper is to present an operating stability diagram for the plasma needle and to establish the optimum working parameters for this device in order to use it as deactivation agent of *E. coli* bacteria.

2. Experimental

The plasma needle was generated using the RF oscillator described extensively elsewhere [16]. The device used for discharge generation and presented later (Fig.1) is a simplified version of that presented and described earlier [14, 15]. The RF power is transmitted from the resonant circuit of the oscillator towards the needle directly, via a BNC connector. No coaxial cable is used to connect the torch to the RF generator; therefore the extra losses due to radiation and dissipation are substantially reduced.

The schematic diagram of the experimental set-up and the picture of the generated plasma are presented in Fig.1.

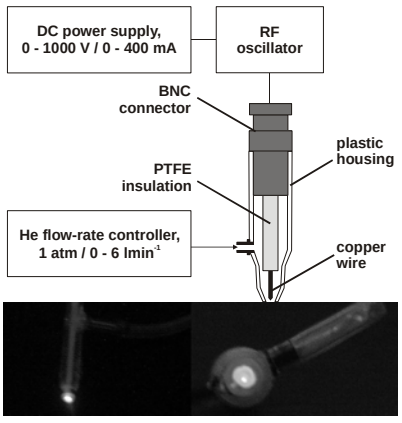

Fig. 1. The experimental set-up for plasma needle generation and the plasma picture at an absorbed power of 2 W and 1 l/min He flow-rate.

The operating stability diagram for the plasma was obtained by following step-by-step the procedure

described by Rezaaiyaan *et al.* [17], Forbes *et al.* [18] and Spencer *et al.* [19]. The data necessary for plotting the operating stability diagram were obtained by performing visual observations on plasma formation and aspect, as function of gas flow-rate (0 to 6 l/min) for different DC supply voltages.

The plasma power was estimated using the subtraction methodology suggested by Horowitz [20] and applied in [16]: the absorbed RF power by the plasma is equal to the difference between the transmitted power from the DC power supply in the presence and absence of the discharge, at the same DC voltage (E_A): $P_{plasma} = E_A (I_{Ap}-I_{Ao})$, where I_{Ap} and I_{Ao} represents the supply DC current intensities in the presence and absence of the plasma

The plasma temperature was monitored via a K type thermocouple connected to a MASTECH MAS 345 digital multimeter and drived by the MasView 1.1 software. The plasma emission spectra were monitored using two Ocean Optics HR4000 spectrometers (one for the 290-430 nm spectral region with a resolution of 0.09 nm FWHM and an other one for 200-1100 nm with a resolution of 0.5 nm FWHM).

The sterilizing capability of the plasma was estimated by exposing E. Coli culture, deposited on a glass plate, to the action of the plasma, both as function of treatment time and gas flow-rate. The cultures were grown on nutrient agar medium for 18 hours at 37°C prior to experimentation. Then were subsequently transferred from the plate under sterile conditions to a saline solution (NaCl 0.9 %) and serially diluted to the required concentration range. A droplet of 100 μl was placed on a glass plate and exposed to plasma action. After treatment, the exposed suspension was transferred to the same medium in Petri dishes and incubated at 37°C for 48 hours prior to determining the surviving number of colony forming units (CFUs).

3. Results and discussion

The operating stability diagram, presented in Fig.2, was obtained by keeping constant the DC supply voltage of the RF oscillator and increasing the He flow-rate up to the value of 6 l/min.

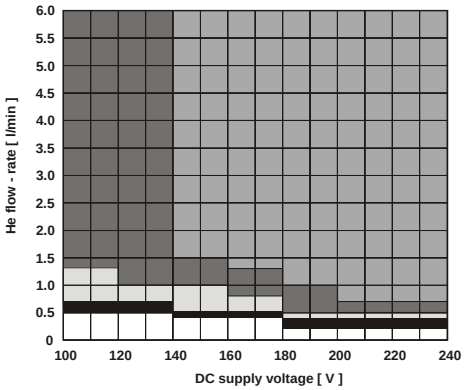

Fig.2 The operating stability diagram.

The diagram indicates that, as function of plasma gas flow-rate and DC supply voltage, there are four zones and three different shapes for the plasma.

Thus, the region denoted "*no plasma*" (coloured in *white*) is defined by He flow-rates below 0.5 l/min for lower DC supply voltage levels (100-140 V), 0.4 l/min for medium voltages (140-180 V) and around 0.2 l/min for higher ones (from 180 V to 220 V). The region coloured in *black* on the stability diagram defines a transition zone, the operating conditions at which the discharge is ignited, the resulting plasma having very small dimensions (little, luminous spark, around 0.5 mm in diameter - "*point-like*" plasma). The increase of the flow-rate or of the DC supply voltage determines a development of the plasma and its transformation into a *ball shaped* discharge (*light grey* on the diagram). At lower flow-rates (below or around 1 l/min) it has a diameter around 1 mm. For higher DC supply voltages (over 180 V) or for high flow-rates (over 1.3 l/min, but voltages below 140 V) the plasma increases its volume, becoming a *well formed plasma-ball* (coloured in *dark grey* on the diagram) with a diameter around 2 mm. The region denoted "*ellipsoidal shaped plasma*" (*medium grey* on the diagram) is defined exclusively by DC supply voltages higher than 140 V (up to 200 V) and He flow-rates from 1.0-1.5 l/min to 6 l/min or by higher voltages (220-240 V) and a much wider gas flow interval (0.7-6.0 l/min).

It has to be mentioned that for this region, by increasing the He flow-rate, the plasma ellipsoid length will increase bit by bit, from 3 mm up to 6 mm, simultaneously with the increasing flow-rate and there is no jet formation.

The absorbed plasma power, as function of He flow-rate for different supply voltages is presented in Fig.3.

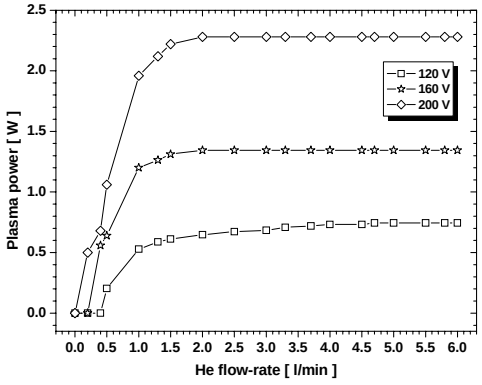

Fig.3 Plasma power as function of He flow-rate for different DC supply voltages

As it can be seen, the plots are in good agreement with the stability diagram. All the plots are showing that the plasma is well formed at starting flow-rate of 0.5 l/min and a saturation tendency starts from 1.0-1.5 l/min (the plasma becomes fully developed). The plasma power as

function of the DC supply voltage, for a He flow-rate of 1 l/min is depicted in Fig.4.

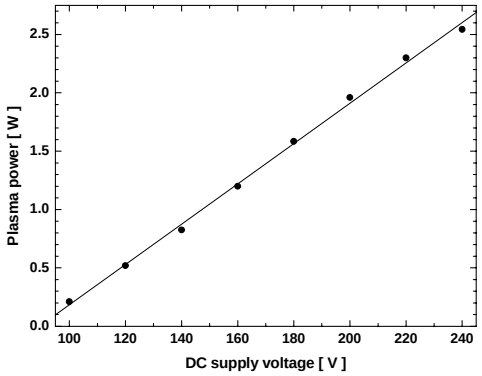

Fig.4 Plasma power as function of DC supply voltages (1 l/min He flow-rate).

The plasma temperature as function of thermocouple-plasma distance is depicted in Fig.5 for several DC supply voltages.

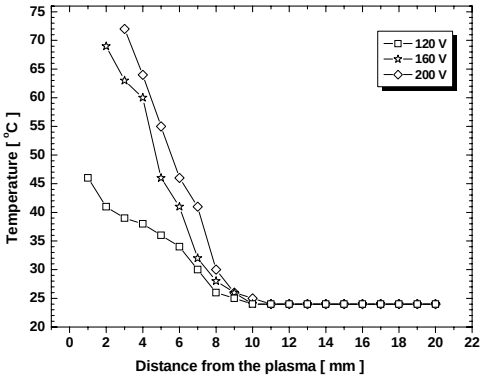

Fig.5. Plasma temperatures for different DC supply voltages (He flow-rate 1 l/min).

As it can be observed, no significant temperature change take place for distances higher then 10 mm from the plasma. As approaching towards the plasma, the temperature will increase as expected up to 45 ^{o}C for lower DC supply voltages (120 V) and above 70 ^{o}C for higher values (160 and 200 V). At high voltages there is the possibility of unwanted phenomena: arc formation. Thus, temperature measurements were not performed for these values below 3 mm from the plasma.

The emission spectrum of the plasma is presented in Fig.6. Studying its composition, the emitted lines and bands could be ascribed to specific excited atoms and molecules.

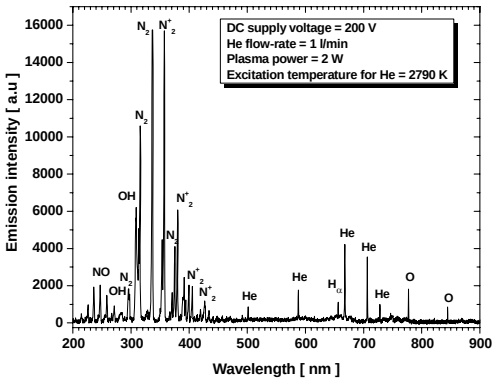

*Fig. 6. **Plasma spectrum***

The He neutral lines could be identified at 501 nm, 587 nm, 667 nm, 706 nm and 728 nm. The excitation temperature for He, determined with the Boltzmann plot method, as function of gas flow-rate or RF power, is situated between 2300 and 3000 K (0.2-0.25 eV).

Since our system is an open-air one, species specific to air will be present in the spectrum, as expected: NO (around 250 nm), the second and fourth positive system of N_2, the first negative system of N_2^+, OH (306 nm) and O_2 (at 777 and 844 nm) and H_α at 656 nm. Because of its weak emission bands, ozone was not detected in the spectrum.

In order to study the microbial deactivation capabilities of the plasma, a droplet of 100 µl E. Coli culture was placed on a glass plate at a distance of 3 mm from the plasma and exposed to its action as function of treatment time or gas flow-rate. The results (average of 5 replicate measurements) and operating conditions are presented in Fig.7 and Fig.8.

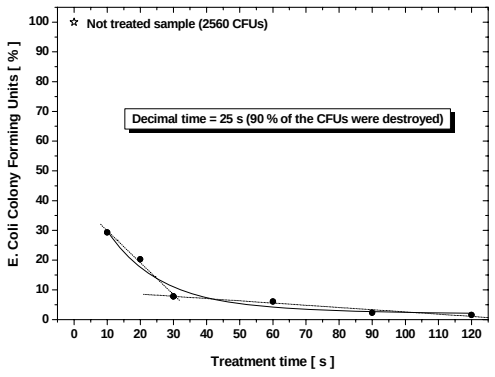

Fig. 7. Deactivated colony forming units (CFUs) as function of treatment time (DC supply voltage 200 V, He flow-rate 1.3 l/min, absorbed RF power 2 W, plasma-bacterial culture distance 3 mm).

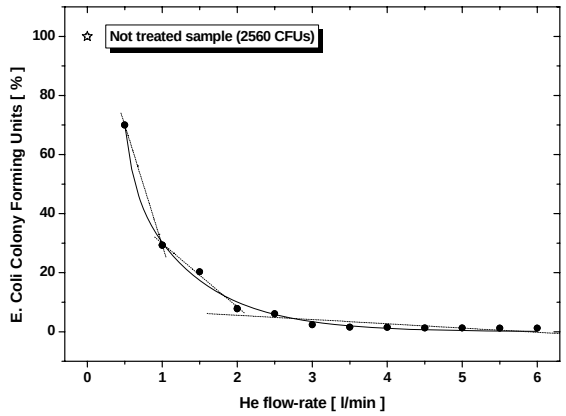

Fig.8. Deactivated colony forming units (CFUs) as function of gas flow-rate (DC supply voltage 200 V, treatment time 30 s, plasma-bacterial culture distance 3 mm).

As one can observe, the plasma is capable of destroying bacterial culture, the overall decimal time (treatment interval necessary to inactivate 90 % of the initial CFUs) is around 25 s. The concentration of surviving units decreases exponentially with time, after 120 s of exposure, almost 99 % of them being deactivated, but the sterilization process occurs with two different velocities. For treatment times up to 30 s the deactivation is more efficient, the killing rate being about 73 CFU/s. After 30 s of exposure the process becomes slower (approx. 2 CFU/s).

In order to establish the influence of the plasma shape, for a constant treatment time of 30 s, the deactivation capability of the plasma was studied as function of He flow-rate. Thus, as expected, there is no deactivation in the ignition zone (point like plasma) and it is weak for the ball shaped plasma. The sterilizing capability of the plasma becomes significant over 1 l/min He flow-rate, when its shape turn to ellipsoidal. The dependency of the survivals shows that by increasing the flow-rate there is no significant change for values higher than 2.5-3 l/min (killing rate of 37 CFU/s) as compared to lower values of the flow-rate (2084 CFU/s when ball shape plasma turns to ellipsoidal and 550 CFU/s for 1-2 l/min He flow-rate).

The effect of the plasma on the bacterial culture was compared with two classic methods: heat and germicidal UV radiation. The results, for a treatment time of 30 s, are presented in Fig.9.

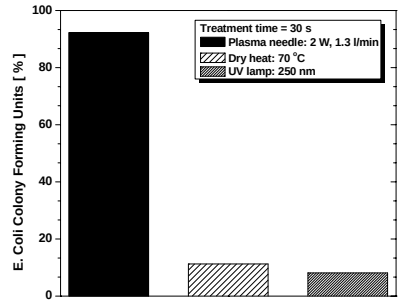

Fig.9 *Plasma deactivation effect versus heat and UV radiation*

As one can see, for small amount of samples, the plasma offers a quick and effective sterilization for short treatment durations where classical methods are known not to be efficient: for a treatment time of 30 s, 92.20 % of the CFUs were deactivated, almost 9 times more than by dry heat (11.25 %) and 11 times more the by UV radiation (8.09 %).

4. Conclusions

The operating stability diagram has four major zones for the atmospheric pressure He plasma needle obtained in our laboratory and it indicates that as function of supply voltage and plasma gas flow-rate there are three different shapes for the plasma (point, ball and ellipsoidal shape). Using this diagram the optimum working parameters could be established for the atmospheric pressure He plasma needle as function of the specific requirements of the performed studies and applications. The plasma temperatures and sterilization power has been demonstrated for an RF power of 2 W and 1 l/min He flow-rate and it was compared with classical sterilization methods (dry heat and UV radiation). The overall onset of deactivation lays around 25 s of treatment time, after 2 min almost 99 % of the CFUs being deactivated.

Acknowledgements

This work was supported by the Romanian Ministry of Education through National Authority for Scientific Research RTD National Programme CEEX under Grant ET 99 (5858 / 2006).

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