Helium atmospheric pressure plasma jet parameters and their influence on bacteria deactivation in a medium

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Abstract

Atmospheric pressure plasmas are becoming relevant in local microbial deactivation and other combined effects of plasmas on living organisms. For this reason, our research was focussed on optimisation of atmospheric pressure plasma jet (APPJ) parameters to complete the deactivation of different bacteria strains in a medium. Different helium APPJ treatments with different discharge parameters were used, such as input voltages and gas flows. To better understand plasma properties behind complete bacteria deactivation at optimised discharge parameters, optical and electrical plasma jet diagnostics were performed, including electrical characterisation of the plasma source, optical emission spectroscopy of the plasma plume and intensified charged coupled device imaging of the discharge behaviour for every set of plasma parameters. Then, the resulting plasma liquid chemistry was assessed to establish the connections between reactive species generated in the gaseous and liquid phases. The most efficient deactivation was found for higher discharge powers and gas flow rates, and that was linked to higher densities of reactive oxygen and nitrogen species, especially hydrogen peroxide and medium solvated charges.

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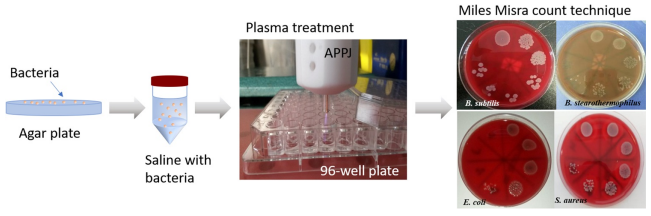

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Helium atmospheric pressure plasma jet parameters and their influence on bacteria deactivation in a medium

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Abstract. Atmospheric pressure plasmas are becoming relevant in local microbial deactivation and other combined effects of plasmas on living organisms. For this reason, our research was focussed on optimisation of atmospheric pressure plasma jet (APPJ) parameters to complete the deactivation of different bacteria strains in a medium. Different helium APPJ treatments with different discharge parameters were used, such as input voltages and gas flows. To better understand plasma properties behind complete bacteria deactivation at optimised discharge parameters, optical and electrical plasma jet diagnostics were performed, including electrical characterisation of the plasma source, optical emission spectroscopy of the plasma plume and intensified charged coupled device imaging of the discharge behaviour for every set of plasma parameters. Then, the resulting plasma liquid chemistry was assessed to establish the connections between reactive species generated in the gaseous and liquid phases. The most efficient deactivation was found for higher discharge powers and gas flow rates, and that was linked to higher densities of reactive oxygen and nitrogen species, especially hydrogen peroxide and medium solvated charges.

1 Introduction

It is well known that some microorganisms, such as bacteria, fungi and viruses, act as pathogens and induce various diseases. Moreover, microorganisms can cause food spoilage and damage to materials such as corrosion of plumbing systems. For these reasons, several conventional sterilisation techniques which lead to complete microbial deactivation or removal have been developed, including heating, filtration, chemical liquid agents and radiation. However, a disadvantage of these sterilisation techniques is that they can be used only on thermally resistant and chemically inert substrates, as those techniques can influence substrate properties [1].

In recent years, non-thermal atmospheric pressure plasmas have been proposed as an alternative to conventional sterilisation techniques. Most frequently reported is sterilisation with atmospheric pressure plasma jets (APPJs) due to their low operating temperatures and cost-effective operation [2–5]. APPJs are suitable for selective treatment of specific substrates as they contain more known inactivation agents without the downsides of conventional sterilisation techniques. Research suggests that reactive oxygen species play the biggest role in bacteria inactivation, but UV radiation, electric field, other reactive species and charged particles also contribute to the process [5–8]. In this way, APPJs represent one of the most promising discharge candidates for different biological applications, including complete deactivation of bacteria [9–12].

This research tested the efficiency of a constructed APPJ on four different bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus stearothermophilus*. *B. stearothermophilus* and *B. subtilis* are spore-forming bacteria and the most commonly recognised and widely used biological indicators for monitoring the effectiveness of sterilisation processes. Spores are dormant bacterial structures, highly resistant to disinfectants and sterilising agents. Spore-forming bacteria are commonly found in processed foods and dairy products [13–15]. These bacteria were tested in order to see how an APPJ affects spore-forming bacteria. Additionally, *E. coli* and *S. aureus,* the most common pathogens in humans and widespread in nature (in hospitals and working and living surroundings), were selected. They are commonly found

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in different environments, contaminating various items, medical tools and food, and can cause hospital infections and food poisoning, as well as medically severe and sometimes fatal infections [16–19]. Moreover, these bacterial strains are known to possibly be multidrug-resistant [20, 21]. In addition, these bacteria tend to form biofilms where bacteria are well protected from the outside agents. It has been shown that plasmas may sterilize even the biofilms as well as planktonic samples [22].

There have been many reports on atmospheric pressure plasma-induced bacteria deactivation and decontamination [23–27]. However, there is a knowledge gap in optimising plasma parameters so that complete bacteria deactivation in a medium can be achieved in the shortest (optimal) times. Therefore, this research focuses on finding the most efficient parameters of a non-thermal helium APPJ as one of the most frequently used sources for deactivating bacteria. For this purpose, various combinations of input DC power unit voltages and gas flows were tested. Appropriate diagnostics were done both on plasma source and discharge, and on medium bacteria were suspended in.

2 Experimental setup

2.1 Preparation of bacteria samples

The deactivation effect of an APPJ, operated with helium as a working gas, was investigated on four different types of bacteria: *B. stearothermophilus* (ATCC No. 7953, *B. subtilis* (ATCC No. 6633), *S. aureus* (ATCC No. 25923) and *E. coli* (ATCC No. 25922). Bacterial cultures were grown overnight on Columbia (COS) agar plates (bioMérieux SA, Marcy l'Etoile, France) at 55 °C for *B. stearothermophilus* and 37 °C for *B. subtilis, S. aureus* and *E. coli.* bacteria were picked up with a loop and resuspended in sterile saline to obtain 0.5 McF (1.5×10^8 CFU/ml) initial bacterial suspension. The concentration was constant in all experiments. 100 µl of these initial 0.5 McF bacterial suspensions was evenly transferred to a 96-well plate with a flat bottom. Bacterial suspensions were exposed to the He APPJ at a constant distance for different exposure times. The samples were treated each time in triplicates.

To determine viable counts and evaluate plasma treatment effects, the Miles and Misra viable count technique on COS blood agar plate (bioMérieux SA, Marcy l'Etoile, France) was used. A 20 µl properly diluted plasma-treated bacterial suspension, as well as a positive (untreated bacterial suspension) and negative control (sterile saline), was placed onto the blood agar plate. This procedure is depicted in Fig. 1. Measurements of the reactive species and pH were also conducted. Reactive species concentrations of NO_2^- and H_2O_2 were measured by a spectrophotometer (UV VIS Lambda 25) via colorimetric assays in sterile saline. The pH measurements were performed by a pH-meter (Sentron®) also in saline.

2.2 APPJ system

Bacteria-containing medium was treated by APPJ source which is designed to be handheld and highly portable, schematic of which is shown in Fig. 2. The portability of the device was facilitated by a small-size custom-made power source which is placed inside a 20 × 12 × 6 cm plastic box including connectors and switches. It was connected to a commercial DC power supply Voltcraft SPS12-12 W-A. The power source output signal amplitude was varied by changing the DC input signal voltage, at discrete voltages 3, 4.5, 6, 9 and 12 V. Based on the DC input voltage, the output signal at 16 kHz was supplied to the jet enabling plasma operation at several high voltages from 1200 to 3500 V (RMS values). The jet had a copper wire, which serves as needle-type powered electrode that was placed inside the glass tube with inner diameter of 2 mm and outer diameter of 4 mm. The tube itself is held by 125 mm long and 26 mm wide Teflon housing, from which it protrudes 8 mm on the one side. On the other side, the tube was connected to gas inlet and Bronkhorst Mass-View MV-194 flow controller. For these treatments, we used He as a working gas at various fixed flows (0.5, 1, 1.5 and 2 slm).

Optical characterisation comprised optical emission spectrometry and plasma imaging by using an intensified charged coupled device (ICCD) camera. Optical emission spectroscopy was performed with an Andor Shamrock 500i spectrometer equipped with iXon Ultra 897 as a detector. An optical fibre was used to receive the emission from the plasma plume and direct it to the entrance slit of the spectrometer. The fibre was positioned at a distance of 5 cm from the jet tube

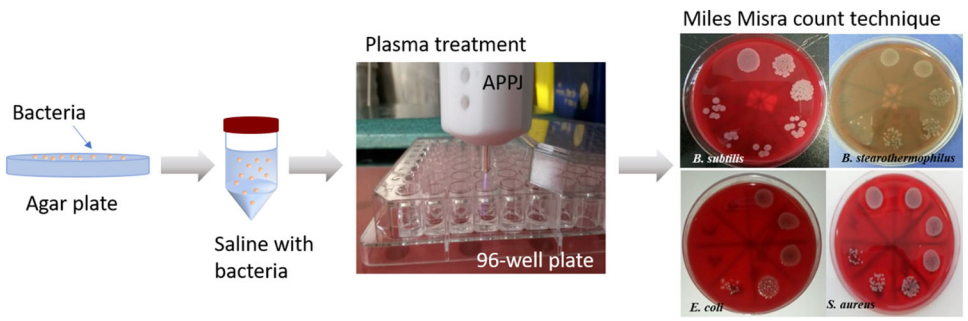

Fig. 1 Schematic representation of the experimental protocol procedure

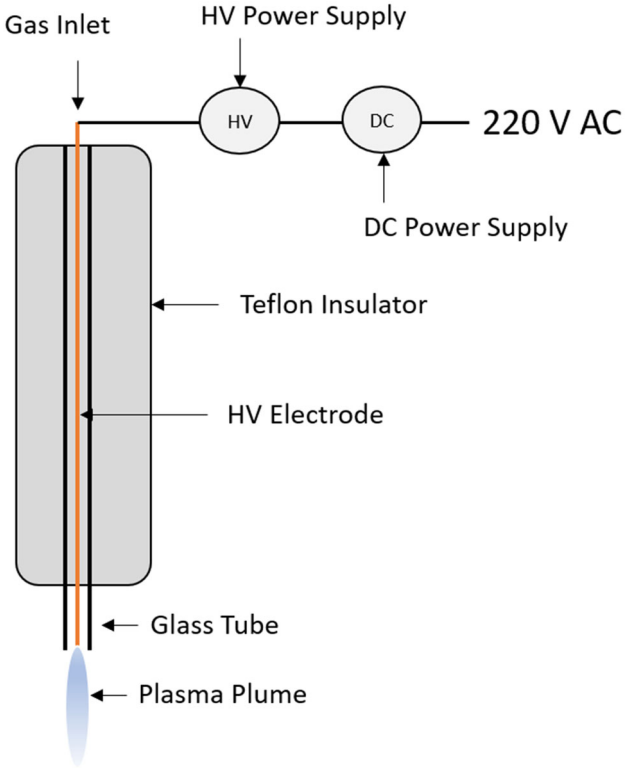

Fig. 2 Schematic representation of an APPJ used in this research

axis to gather the light coming from the whole channel volume. Recording of the spectra was performed for exposure times of 100 ms and with averaging of 10 spectrum acquisitions. Thus, obtained results represent space- and time-averaged emission from the plasma. Plasma imaging was performed with an Andor iStar ICCD camera DH334T-18U-03 equipped with a photographic objective. Images were taken in single-shot mode with an exposure time of 20 ms. Furthermore, electrical characterisation was performed by measuring the average power given to the jet. Voltage and current on the powered electrode were measured before the APPJ with an oscilloscope (Rigol DS1102E), high voltage probe (Rigol RP1018H) and current monitor (Pearson 8590C).

Estimation of saline solution evaporation during treatments was performed to evaluate changes in the treatment conditions throughout the experiments. For the longest treatment times, the highest DC supply voltages and He flow of 2 slm, the evaporated solution volume from the 96-well plate was not more than 50 µl. This change in volume caused a maximum liquid level reduction of 1.2 mm, thus increasing the distance between the plasma jet and the liquid surface. However, these changes did not drastically influence plasma properties, and these maximum values were reached only for the longest treatment times and plasma powers. For most treatment conditions, volume changes fell within the experimental error of transferring the liquid volume into the plate.

3 Results and discussion

3.1 Bacteria deactivation

At first, bacterial suspension control samples were exposed only to helium gas flow with rates of 0.5, 1, 1.5 and 2 slm, without plasma and with no voltage applied, for the same duration as required for deactivation using the plasma. The obtained results exhibit no difference in bacteria viability (Fig. 3) compared to the untreated samples (positive control; PC), which confirms that helium alone is insufficient for bacteria deactivation. The effects of the APPJ were then further tested for all bacteria and analysed with a quantitative and informative approach, which involved dynamical studies of bacterial growth after treatments. Typically, survival curves were determined as the numbers of colony-forming units (CFUs; surviving culturable bacteria as a function of plasma treatment time). However, to limit the presentation, only complete bacteria deactivation, achieving sterility of the medium, is shown in Fig. 4.

Furthermore, Fig. 4 presents the time needed for complete deactivation of *E. coli*, *S. aureus, B. stearothermophilus* and *B. subtilis* within a medium, exposed to a He APPJ generated with different DC input powers and gas flows. If there are no data shown for a specific set of parameters (usually 3 V and 0.5 slm), the bacteria were not completely deactivated within the maximum treatment time of 240 s used in experiments. In most cases, it was found that *E. coli* was deactivated faster than other bacteria, proving to be a less plasma-resistant strain. In this case, the highest treatment time was 180 s for deactivation under the lowest gas flow of 0.5 slm, which typically did not prove very efficient. *B. stearothermophilus* strains proved the most resistant to plasma treatments. Surprisingly, the lowest flow rate deactivation curves for 0.5 slm are very similar for all types of bacterial strains, except a small deviation with *E. coli*. This indicates that the APPJ generated at these conditions and its consequent reactive oxygen and nitrogen species (RONS) chemistry within the medium are similar (although, moving to higher flow rates, the chemistries and deactivations changed significantly). The trend follows the same patterns, where 0.5 slm is the least, and 2 slm is the most efficient, which are plasma properties connected to its subsequent interaction. The exception to this general rule is *B. stearothermophilus*, the most thermally stable and resistant strain, which seems to deviate from the rule. In this case, the most efficient chemistry for deactivation is at 1 slm. Chemical analyses of the medium chemistry elucidate the reasons for this behaviour in the following paragraphs.

From the perspective of the DC input voltage parameter used for jet discharge, the general rule is: the higher the energy input into discharge, the faster the deactivation of bacterial strains. However, it seems there is a minimum level at which the jets are efficient. It was found that an input DC voltage of 3 V was not sufficient

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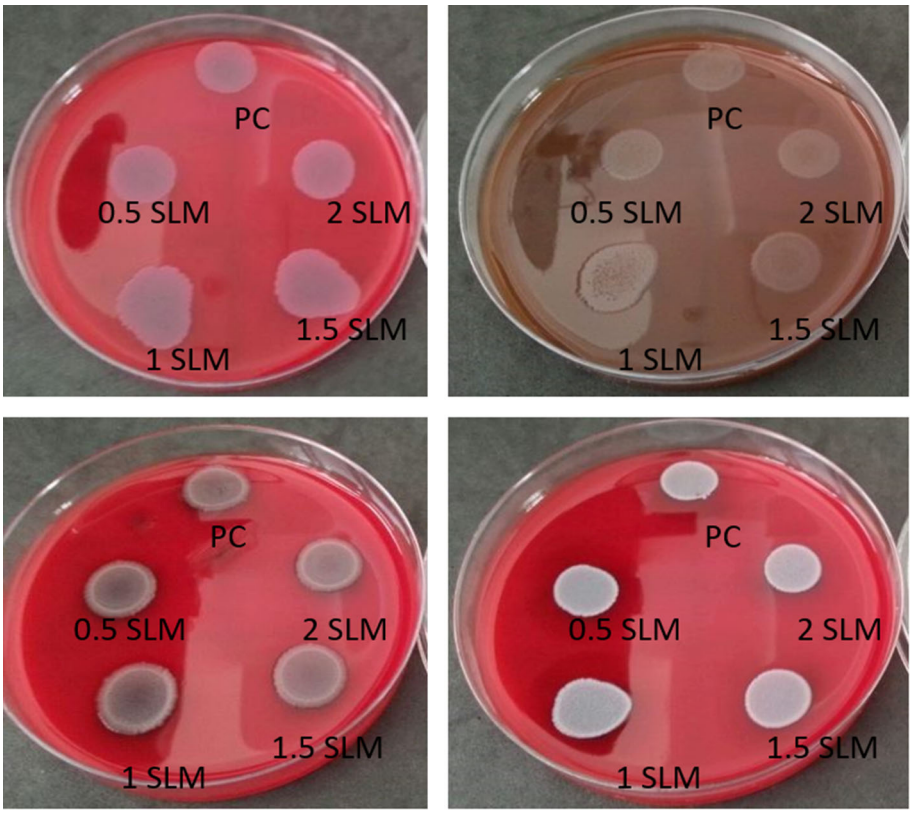

Fig. 3 Effect of gas flow-only (no plasma) treatment of bacteria **a** *B. subtilis,* **b** *B. stearothermophilus,* **c** *E. coli* and **d** *S. aureus* exposed to 0.5, 1, 1.5 and 2 slm compared to the positive control (PC) by Miles and Misra plate counting

to deactivate most bacteria strains even for the highest gas flow and treatment times because the plasma plume was the shortest and was not in direct contact with the substrate. If deactivation of the bacteria strain was achieved, then the treatment time was significantly prolonged. Therefore, the results indicate that He APPJ is most efficient at bacteria strain deactivation with higher applied power and higher gas flows, considering marked limits in discharge parameters and experimental constraints. We do not reach conditions where additional heating would produce thermal necrosis (40 °C) in the covered range of powers. While increasing efficiency with power is expected as for the flow, one could expect that beyond some point, further increasing of the flow may reduce efficiency by affecting the chain of plasma chemical events needed to produce the radicals that cause sterilization.

3.2 Chemical analysis of reactive species of saline medium treated by APPJ

To explain the obtained results for bacterial deactivation in a medium, the initiated medium chemistry was investigated, determining RONS species, especially H_2O_2 and NO_2^- concentrations of the APPJ treated saline solution. Immediately after treatments, we performed measurements of pH changes. These parameters are known to influence the viability of bacterial strains significantly, as marked by numerous reports [28–31].

pH measurements were made under the same conditions as for reactive species measurement. The influence of different He plasma parameters (different gas flow rates of 0.5, 1, 1.5 and 2 slm and different input DC powers of 3, 4.5, 6, 9 and 12 V) on pH value was systematically measured. A pH value decrease was observed during the plasma treatment for most cases (Fig. 5). These decreasing trends featured an initial drop and then a steady decrease. An exception was 1 slm, which had an increasing pH trend for input DC voltage of 3 V. This could be explained by the fact that the plasma jet did not touch the surface of the liquid, and in this case, the chemistry of the medium was different than in other cases.

Reactive species concentrations of NO_2^- and H_2O_2 were determined after plasma treatment of saline; 50 µl of sterile saline was placed in the 96-well plate with a flat bottom. The distance between the bottom of the well and the APPJ orifice was 15 mm, as for the treatment of bacteria, and was kept constant during the treatment. The results are presented in Fig. 6, and the results are obtained with only the parameters yielding the most efficient plasma treatment—input voltage of 12 V and a flow rate of 2 slm. An expected, steady increase of H_2O_2 concentrations was observed for increasing treatment time. In contrast, the concentration of NO_2^- increased until 30 s, where it reached its maximum value and then started decreasing. The concentration dropped to zero after 120 s. This could be explained through decreasing of the pH value during the treatment. NO_2^- is very sensitive to low pH values, which is the cause of its decomposition or transformation into other compounds [32].

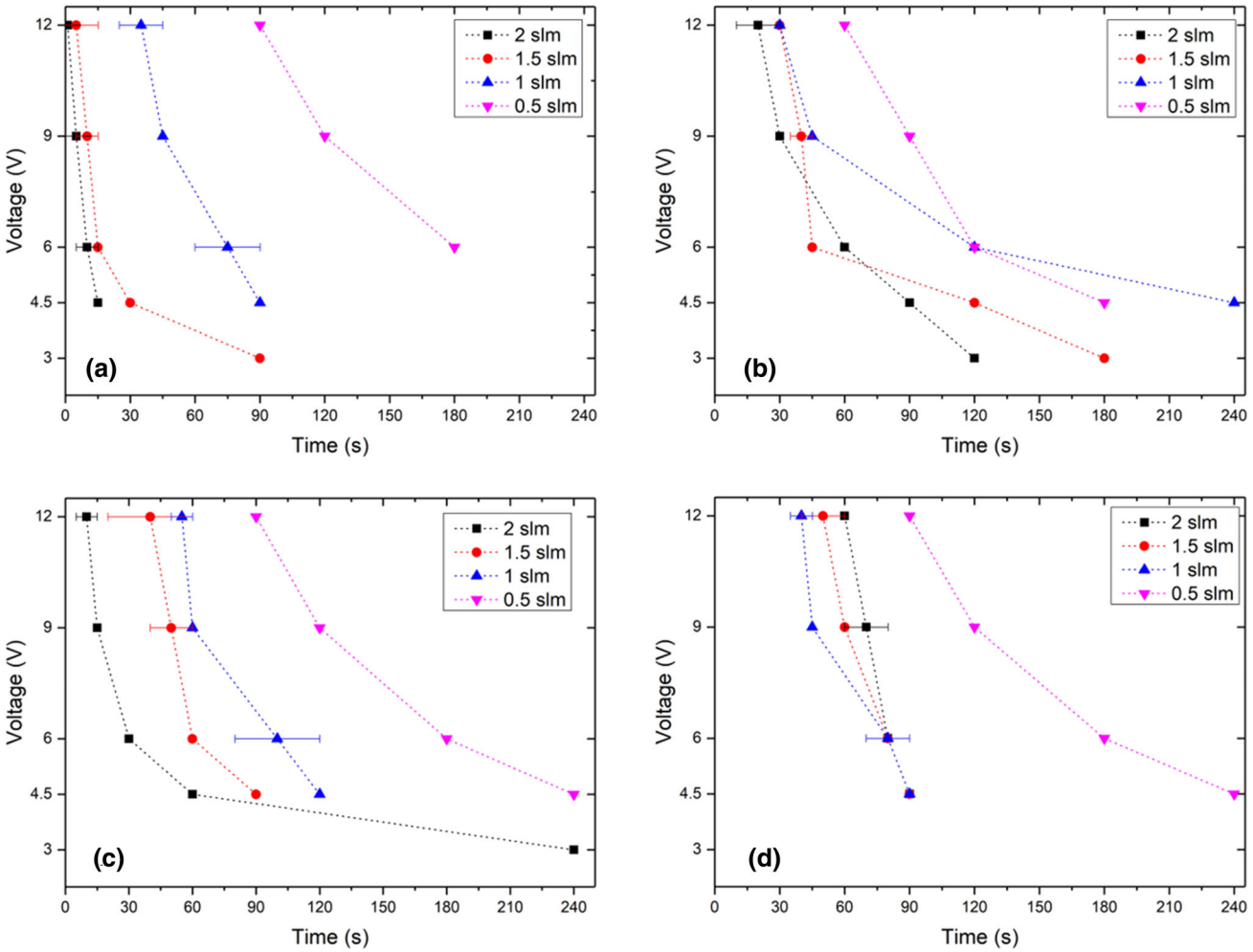

Fig. 4 Points of complete bacteria deactivation (the point at which the initial CFU/ml concentration drops to zero) **a** *E. coli,* **b** *S. aureus,* **c** *B. subtilis* and **d** *B. stearothermophilus* exposed to He APPJ generated with powers of 3, 6, 9 and 12 V, and flow rates of 0.5, 1, 1.5 and 2 slm

3.3 Diagnostics of the plasma source

In order to analyse properties of the plasma used for treatments, we performed diagnostic experiments at the same conditions as when treating media with bacterial strains. Due to safety, a saline medium was used without bacteria for these measurements.

Power measurements were made via electrical characterisation, where the average power (P_{avg}) input into the jet was measured. This was calculated over 30 periods of current and input voltage as:

$$P_{\text{avg}} = \frac{1}{30T} * \int_{t1}^{t2} P(t)dt,$$

where T is oscillation period, and $P(t)$ is instantaneous power in every moment t calculated as $I(t) * V(t)$ from the beginning t_1 and end t_2 of 30 periods. The measurements were performed on the electrode before the plasma jet coming out of the tube and at two gas flow rates of 1 and 2 slm. The calculated values present an average power that the power source gives to the plasma jet (Fig. 7 left axis) and represents the 'real' power input into plasma. In order to link electrode voltage and power and to facilitate comparison to the other experimental data, we calculated V_{RMS} values as a function of input DC voltage (Fig. 7 right axis). The RMS values were calculated for 30 periods assessing several $V(t)$ signals at the same DC voltage in order to estimate differences. The measurements were performed on the electrode at two gas flow rates of 1 and 2 slm. It was observed that the power was not influenced by the gas flow rate but was instead dependent on the DC input voltage and provided powers in the range of 0.1–1.5 W. The power that is transferred from plasma to the treated samples is somewhat lower than calculated power since part is always lost.

Optical emission spectroscopy was used as a plasma diagnostic tool. A typical spectrum of He discharge of an APPJ at gas flow rate of 2 slm where a jet was positioned above the saline solution target is presented in Fig. 8. The spectrum was recorded in a wide range

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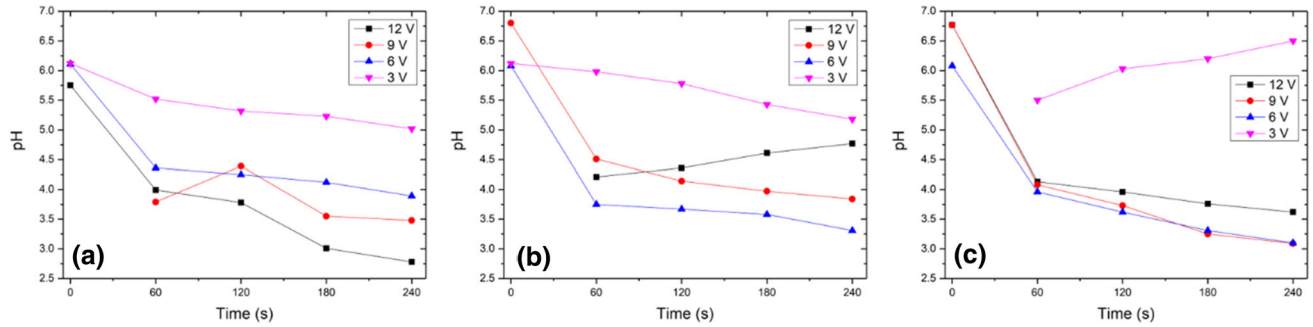

Fig. 5 pH values of He APPJ treated saline for gas flow of **a** 2 slm, **b** 1.5 slm and **c** 1 slm

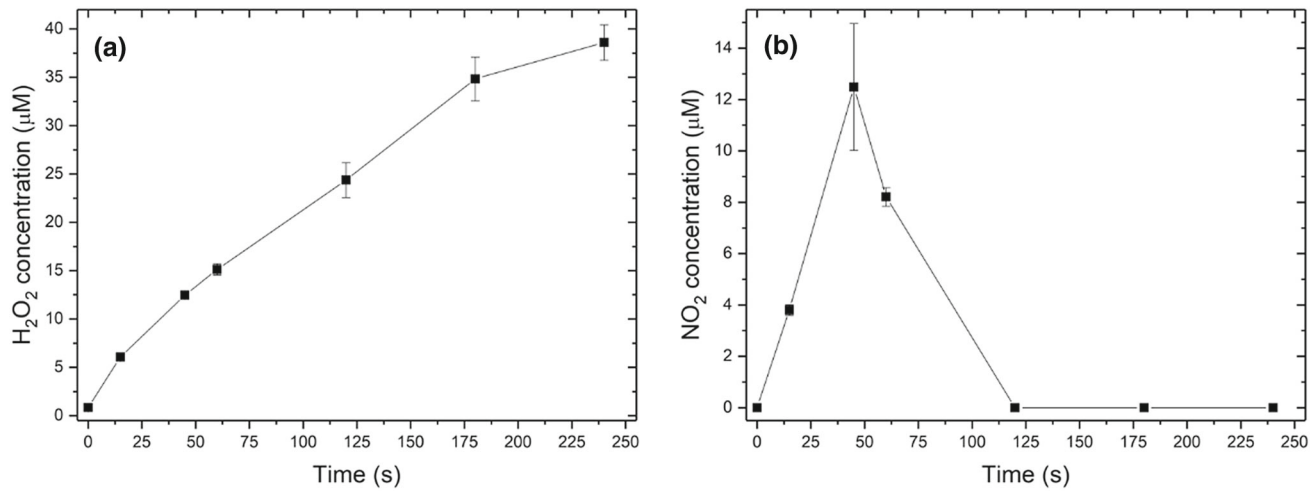

Fig. 6 Concentration of reactive species: **a** H_2O_2 and **b** NO_2^- with respect to treatment time

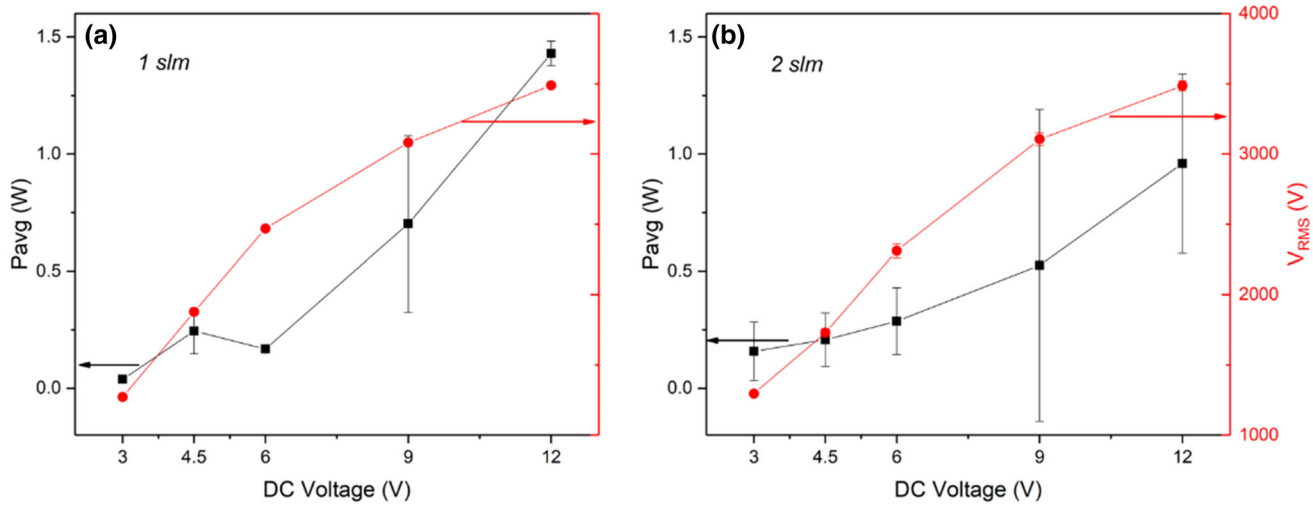

Fig. 7 Average input power to the plasma jet for He gas flows of **a** 1 slm and **b** 2 slm

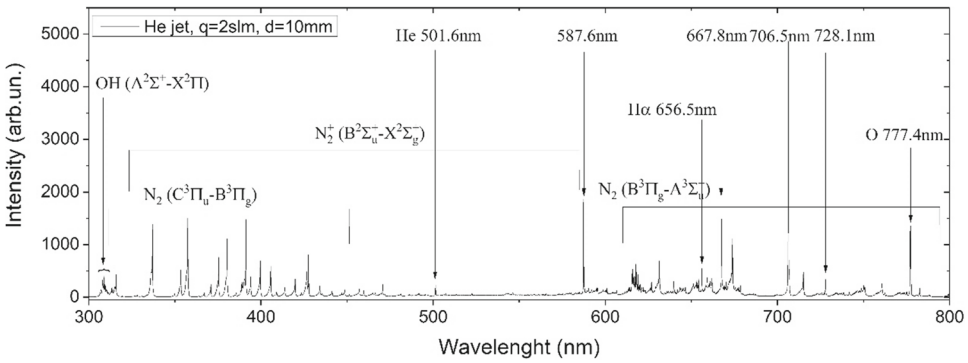

Fig. 8 Optical emission spectroscopy of the He APPJ with a characteristic spectrum generated during the treatments above the liquid medium

of wavelengths, between 300 and 800 nm. The characteristic spectrum of excited species has already been assessed for this kind of plasma jet [33–35]. The most intense lines came from the molecular OH (A–X) band, atomic lines of He and O, and molecular bands of N_2; the second positive system (SPS) and the first positive system as well as from the nitrogen ion—the first negative system (FNS) [36, 37]. Excited He atoms were produced from the ground state neutrals in the working gas used in the system. At the same time, OH and N_2 bands and atomic O lines and Hα line were present since the experiments were conducted in an ambient air (with some humidity) and in contact with the saline solution. Neutral species from the surrounding air were mixed with the helium flow and therefore participated in gas phase reactions induced by plasma [38, 39]. In contrast to the case where solid NaCl was treated [40], the spectrum obtained with saline solution did not show any additional lines from Na (or Cl). This suggests that these species were not excited in the gas phase above the water for the plasma source to excite them.

Additional analysis regarding line intensity was performed on specific atomic and molecular lines for different DC input voltages used in the experiment (3, 4.5, 6, 9 and 12 V) at two gas flows (1 and 2 slm). The intensities at different discharge parameters with two emission lines from N_2 SPS (337.1 nm and 315.9 nm), head line from FNS N_2^+ (391.3 nm), the strongest molecular OH line (309 nm), Hα (656.6 nm) and O atom line (777.4 nm) are presented in Fig. 8, while He line (706.5 nm) intensities are depicted in Fig. 9. All line intensities are normalised to the same recording conditions and corrected for spectral efficiency of the system, thus allowing direct intensity comparison between different lines. The position of the jet and the distance to the bottom of the 96-well plate were the same as for the treatments of bacteria. There was an increase in intensities for all observed lines when the source power (DC voltage) was increased. The increase of He flow had a minor influence on line intensities, resulting in a somewhat higher line intensity. In all cases, there was a stronger or weaker 'jump' between the emission intensities recorded for 6 V and 9 V. This change in peak values occurred due to the change in plasma regime since, as observed with the naked eye, the plasma channel did not connect to the surface of the saline until the 9 V were reached [41]. Therefore, the intensities recorded for voltages below 9 V can be regarded as free-standing jet cases, while for the voltages of 9 V and 12 V, plasma plume was in contact with the liquid surface.

The highest line intensities belong to the mainline of N_2 SPS, and these intensities have pronounced increments between 6 and 9 V input voltage (Fig. 9a and b). The second strongest line of the same band has a much lower increase in intensity. However, excitation of both of the excited levels in N_2 probably happened through electron collisions with the ground state or excited N_2 molecules [35, 42]. The increasing line intensity tendency is in accordance with the dependence observed with similar jet configurations [34]. The intensity of the strongest of FNS N_2^+ lines at 391.3 nm also increased with DC voltage, yet much less than the 337.1 nm line (Fig. 9a and b). This line comes from the excited state of N_2^+ ions that were efficiently produced in the Penning ionisation process, involving He metastables [43] and the direct electron impact ionisation process [42]. Consequently, an increase in He flow made the emission of the 391.3 nm line rise. On the other hand, lines from the OH band and Hα came from dissociation of water vapour molecules in plasma [35, 44]. In this jet configuration, the amount of water vapour present in the surrounding air was sufficient to produce several excited species of OH and H visible in the emission spectrum. An increase in the He flow and discharge voltage resulted in the increase of OH emission intensity (Fig. 9c and d), which has been observed before [34, 45]. The atomic O (777.4 nm) line exhibited similar behaviour. Production of both OH and O species is important when it comes to the treatment of bacteria.

The intensity trend of the He line at 706.5 nm was similar to that of other spectral lines and is presented in Fig. 10. As expected, line intensity was observed to increase when we increase either working gas flow or DC input voltage. This He line is the most intense compared to other lines observed in the spectrum (Fig. 10). The result is due to amount of He and its mixture with air present in the plasma plume.

Observing all line intensities analyzed here, one can conclude that within the range of voltages varied in the experiment, there is a steady increase of the line intensity with increase in applied voltage. This reflects a fact

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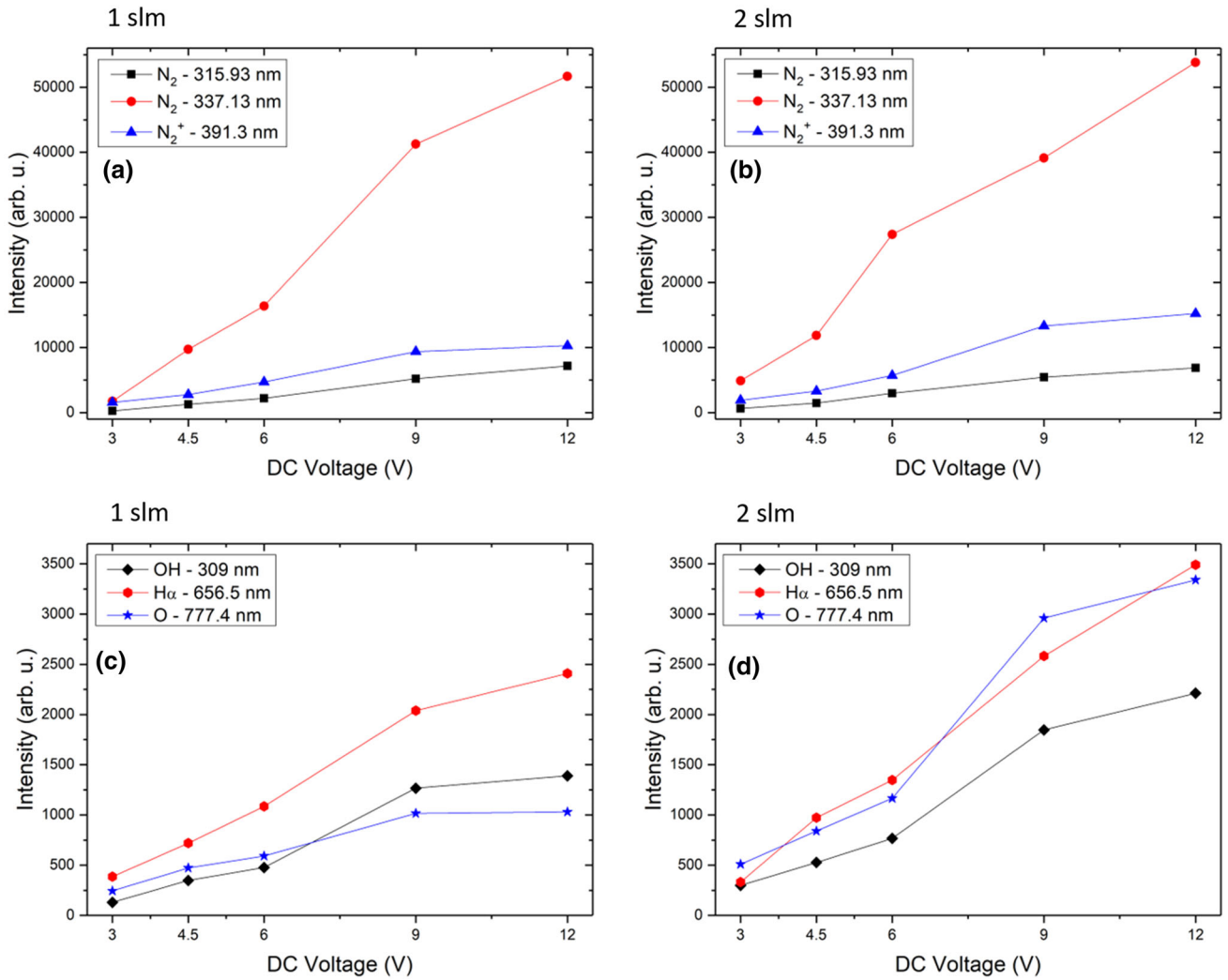

Fig. 9 Optical emission spectroscopy: intensities of certain atomic and molecular lines of nitrogen (**a** for 1 slm and **b** for 2 slm), and hydrogen, oxygen and hydroxyl **c** for 1 slm and **d** for 2 slm)

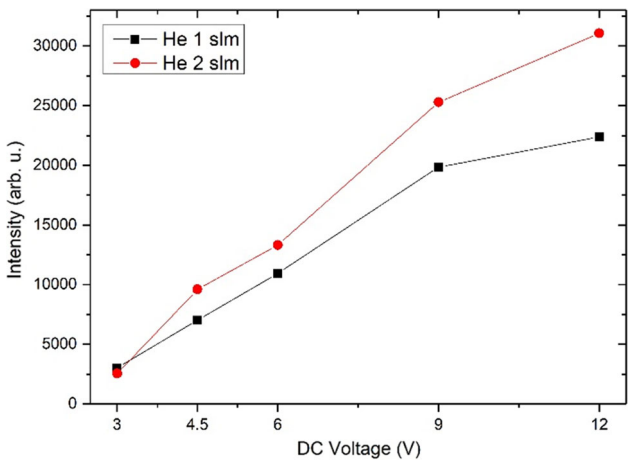

Fig. 10 He 706.5 nm line intensities for different gas flow and voltages

that for all lines, i.e. processes related to specific emission, concentration of excited species continuously rises with voltage increment, without any abrupt changes. Hence, we can say that both voltage and flow changes applied here do not influence plasma chemistry but only concentration of species involved in the processes.

Discharge imaging can provide information about the way plasma plume forms and how it propagates between electrode and bacteria-containing medium. Additionally, a relative abundance of active species can be roughly assessed through emission intensity as brighter intensity corresponds to more emitting particles. For this, ICCD imaging was employed with time-averaged images of the streamer structure obtained for all He flows and DC input powers. Typical results are presented in Fig. 11 for 1 slm and 2 slm, at only the lowest (3 V) and highest (12 V) DC voltages used in the experiments. Similar to optical emission measurements, the jet position and its distance to the liquid surface in

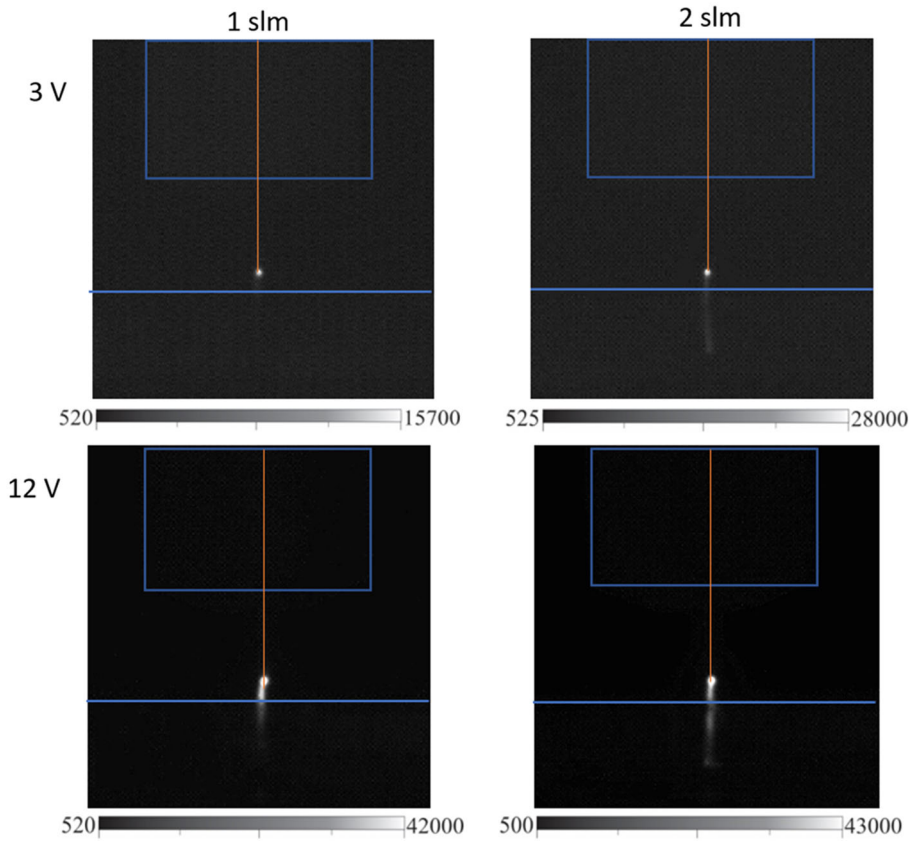

Fig. 11 Images of the discharge structure at different flows and DC voltages from the power supply. A thin vertical line represents a pin electrode protruding from the body of the jet (rectangle shape). The horizontal line in the lower part of the images signifies the upper edge of the 96-well plate

the 96-well plate were the same as for bacteria treatments. For all conditions, a ball-shaped bright plasma was visible on the tip of the pin electrode with a plasma plume extending towards the target. For the lowest DC voltage employed, a weak plasma channel existed only for the 2 slm flow of He. Obviously, for $V_{\mathrm{DC}} = 3$ V and 1 slm of He, the field attained at the electrode with these power supply conditions was not enough to achieve sufficient ionisation in the whole volume between the jet and sample surface. At the highest power, i.e. DC voltage of 12 V, a streamer-like plasma channel with strong emission bridged the distance from the electrode tip to the liquid surface. After processing all images recorded by subtracting the background intensity level, it was determined that in all applied conditions, except for 3 V at 1 slm, the plasma plume reached the liquid surface, meaning that streamer length in this range of conditions did not depend on either voltage or helium flow. This indicates that the medium chemistry of bacteria deactivation depends on streamer forming behaviour, which almost doubles the procedure's efficacy.

4 Conclusion

To optimise bacteria deactivation in media and obtain sterilisation with plasmas, a parameter study involving a large number of experiments using different plasma conditions was performed. It included monitoring the viability of different bacteria strains with respect to several plasma diagnostics measurements. This research clearly shows non-thermal helium APPJs' ability to deactivate four standard strains of bacteria used in such experiments. The deactivation effects of the plasma jet were significant and dependant on the bacterial strain, exposure time and plasma configuration (gas flow rate and input DC power unit voltage). The obtained results are expected and indicate that *E. coli* is deactivated faster than other strains. Generally, all bacterial strains—*E. coli*, *S. aureus, B. stearothermophilus* and *B. subtilis*—follow the same deactivation trends. The only discrepancy is in the optimal parameters for deactivation of *B. stearothermophilus*, where optimal deactivation is reached at lower flow rate levels. This might be because of the bacterial strain's properties and its response to the changing environment by interacting plasma. The interaction of plasma and bacteria suspension (saline) was twofold—it changed the concentration of reactive species and pH in the solution with bacteria. This RONS species (He, O, N, H, photons) generated in the gas phase and high-energy electrons and ions were interacting with the liquid. As a result of the combined action of produced reactive species and chemical reactions, which also influenced the pH in the liquid phase, increased bacteria deactivation efficacy. Combining all the chemically initiated processes managed to sterilise given bacterial strains in a medium in fairly short treatment times, maximum

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efficacy was observed at high flow rates and DC input powers. More power transferred into the plasma shortens the deactivation process. Increasing the flow rate from 0.5 to 2 slm also shortens the inactivation process as reactive species concentration in the gas phase rises.

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Author contributions

UC and ZLP conceived and planned the experiments. NH, MM, DV and MĐ performed plasma treatment of bacteria and liquid chemistry analyses along with the interpretation of those results. NŠ, KS and AJ performed plasma diagnostics and electrical characterization along with interpretation of those results. AJ wrote the original draft, and all co-authors helped with manuscript revision.

Declarations

Data availability statement This manuscript has no associated data or the data will not be deposited. [Authors' comment: . . .].

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