Sporicidal properties from surface micro-discharge plasma under different plasma conditions at different humidities

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Abstract
In the current study, bacterial endospores of Geobacillus stearothermophilus are exposed to the surface micro-discharge plasma for 5 min and the humidity and power consumption are varied. At the low humidity of 5.5 ± 0.5 g m⁻³, almost no sporicidal effect (<0.5 log) is observed. At the high humidity of 17.9 ± 0.6 g m⁻³, the spore reduction increases monotonically up to 3.5 log with increasing power consumption. At a humidity of 10.4 ± 0.6 g m⁻³, the spores are inactivated in a limited range of power consumption with a maximum reduction of ∼2.5 log. The survival curves show a single-slope decrease of the spores. The contribution of heat and UV to the sporicidal effect as well as the inactivation of spores by the short-lived species from the plasma are ruled out. The concentration of ozone, one indicator for the long-lived species, is measured and no correlation with the sporicidal effect is found. In conclusion, water-related reactive species, e.g. hydrogen peroxide, appear to be responsible for the sporicidal effect under the investigated conditions. Furthermore, condensation of water at high humidity enables the plasma-activated water containing both long-lived and short-lived reactive species to contribute to the sporicidal effect.

Keywords: plasma sterilization, dielectric barrier discharge, surface micro-discharge, spores, cold atmospheric plasma
1. Introduction

Research on cold atmospheric pressure plasma (CAP) for biomedical applications has been drawing increasing attention in recent decades [1–5]. Here, the background gas, e.g. the ambient air, is weakly ionized at atmospheric pressure, producing electrons, ions, excited particles, UV radiation and heat. By diffusion and/or external gas flow, reactive species produced in plasma are transported to objects, e.g. contaminated surfaces, and inactivate pathogenous microorganisms. The antimicrobial effect of the CAP has been demonstrated by a number of studies and the scope of its application extends over the sterilization of tools and devices in medical facilities, the food packaging and the aerospace industry. CAP can be applied for the in vivo disinfection on wounds and enhance the wound healing process [6–8]. In regard to the alarming dimensions of nosocomial infections, i.e. infections acquired in medical and healthcare facilities, and for the growing challenge of basic disinfection for medical personnel, CAP offers a promising alternative to conventional disinfection and sterilization methods, e.g. dry or moist heat and toxic chemicals [1, 9, 10]. Maisch et al successfully demonstrated the inactivation of antibiotic-resistant bacteria, e.g. methicillin-resistant Staphylococcus aureus (MRSA) by CAP [11]. The application of CAP offers the possibility of inactivating pathogenous microorganisms without harming the user or damaging treated material with heat or chemicals [12, 13].

One possibility to generate CAP is given by surface micro-discharge (SMD), designed and described by Morfill et al based on the dielectric barrier-discharge (DBD) [1]. The antimicrobial effect of SMD has been verified by several former studies; different vegetative bacteria strains [1, 11, 14], biofilms [15] and viruses [16] were successfully inactivated. Moreover, SMD treatment has sensitized brain-cancer cells to chemotherapy [17], has induced the senescence of melanoma cells [18] and has enhanced the wound-healing process [19]. The selectivity between the prokaryotic and eukaryotic cells in the inactivation process by SMD plasma was demonstrated by Welz et al and Boxhammer et al. Here SMD was applied to mucosal tissues and to V79 Chinese hamster cells for up to 2 and 4 min, respectively, and no mutagenic response was observed [20, 21]. Bacterial endospores are regarded as the most resistant terrestrial life form due to their multilayer inner structure (coats, cortex and inner membrane), the low water content in their cores, the capability of DNA repair, and the dormant state with no metabolism and proliferation; they can even survive in space for long periods of time [22–28]. Due to their high resistance to environmental stresses such as heat, desiccation, radiation and toxic chemicals, bacterial endospores are commonly used as biological indicators in sterilization and decontamination processes. The sporicidal effect of SMD plasma has been demonstrated by Klämpfl et al and Shimizu et al using diverse strains of bacterial endospores [14, 29]. A reduction of up to 5–6 log was reached after five to several tens of minutes depending on the distance between the point of plasma generation and the spore samples.

The mechanisms of the inactivation of spores by nonthermal plasmas are not fully understood yet. Several studies have reported successful inactivation of bacterial endospores using a nonthermal microwave plasma setup at low/reduced pressure and have concluded that the sporicidal effect was mainly due to UV irradiation [30–32]. Raballand et al used the particle beams of argon ions and oxygen atoms in order to mimic Ar/O2 plasma at low pressure [33]. They observed the inactivation of Bacillus atrophaeus and Aspergillus niger spores only when both particle beams were active and suggested chemical sputtering as the main mechanism. At atmospheric pressure, it is the prevalent opinion that UV radiation does not contribute to the
sporicidal effect of plasmas [4]. However, Boudam et al found a narrow range of parameters for DBD at atmospheric pressure where the spores were inactivated by UV [34]. Heise et al successfully inactivated B. subtilis and A. niger spores by up to 6 log within several seconds using a combination of an excimer lamp and DBD [35, 36] and concluded that UV radiation was the main mechanism for the sporicidal effect. Hähnel et al successfully inactivated B. atrophaeus spores using DBD with negligibly small doses of UV radiation [37]. They varied the humidity in the treatment chamber and suggested that OH radicals were the main responsible species for the sporicidal effect. Trompeter et al inactivated B. subtilis spores using DBD with a variety of feed gases [38]. They explained the inactivation of spores by UV radiation as well as by reactive oxygen species (ROS) and water-related radicals. Dobrynin et al observed reduction of B. cereus and B. anthracis spores by DBD plasma and mentioned the combined effect of UV and reactive neutral species [39]. Furthermore, they reported that the addition of ethanol vapor to the plasma resulted in enhanced sporicidal effects due to the higher yield of OH radicals and H₂O₂.

It is well known that DBD is able to produce ozone and can be used as an ozonizer, e.g. for the purification of water [40–42]. Mahfoudh et al demonstrated in a comparative study that dry ozone can kill spores, yet is less effective than humidified ozone [55]. They further reported that the spore inactivation efficiency of ozone was dependent on the spore type and the substrate. SMD, a modification of DBD, is capable of generating ozone as well. In a former study, Shimizu et al showed that there are different shapes for the ozone evolution depending on input power [43]. In the low input power range, the ozone concentration increased with increasing input power until reaching ∼100 mW cm⁻². A further increase of the input power resulted in the depletion of ozone, and its concentration dropped to zero within tens of seconds after plasma ignition. They observed a strong correlation between the reduction of the Escherichia coli bacteria and the ozone concentration and suggested ozone as one major bactericidal agent. Recent studies performed by Pavlovich et al and Jeon et al supported this conclusion [44, 45].

In the current study, the sporicidal efficacy of SMD plasma was investigated under different humidity and plasma conditions using the bacterial endospore of Geobacillus stearothermophilus ATCC 7953 as biological indicator. The spore samples were treated by the SMD plasma in a closed volume at atmospheric pressure. As discussed later, the contribution of UV radiation and heat to the sporicidal effect was ruled out. In addition, the impact of short-lived reactive species (electrons, ions, atomic oxygen, singlet oxygen, etc) can be neglected due to the distance between the electrode and the sample surface. The neutral, long-lived reactive species were considered mainly responsible for the inactivation of the bacterial endospores by the SMD plasma, and ozone was chosen as a benchmark. The power consumption was varied in a range from 0.5 W to 14 W in order to apply different chemical components as described above [43, 46]. The ozone concentration in the closed volume was measured via optical absorption spectroscopy. The plasma treatment of the spore samples and the ozone measurements were performed under three different humidity conditions. The sporicidal efficacy was compared among different power consumptions (i.e. different ozone concentrations) and at different humidities in order to optimize the SMD for the inactivation of bacterial endospores. The obtained results and possible mechanisms are discussed.
2. Experimental setup

2.1. Plasma generation

The setup of the SMD used in this study is illustrated in figure 1. A high-voltage powered planar electrode, a dielectric and a grounded perforated plate were laid together with no gap. The planar electrode was made of aluminum with a circular shape of 30 mm in diameter. The dielectric was made of Al₂O₃ with a thickness of 0.75 mm. The grounded mesh electrode was a perforated stainless steel plate with 1.5 mm thickness, 5 mm hole diameter and 7 mm lattice spacing as shown in figure 2(a).

High voltage with a sinusoidal waveform was applied to the planar electrode using a function generator (HM8150, HAMEG Instruments GmbH, Mainhausen, Germany) and an amplifier (PM4015, Trek Inc., Lockport, USA). The applied voltage was fixed at 6.8 kVpp. The applied frequency was varied between 100 Hz and 10 kHz. Figure 2 shows the photographs of the SMD electrode without and with plasma using 3 kHz. When the planar electrode was powered, a large number of filamentary discharges with a length of up to a few millimeters formed between the dielectric and the perforated electrode along the circular openings. The current between the mesh electrode and the ground was monitored using an inductive current.
meter (Model 6585, Pearson Electronics Inc., USA). Figure 3 shows the input voltage and the current at 3 kHz. The current signal is shifted in phase from the input voltage and showed a large number of spike-shaped currents, each corresponding to a single micro-discharge. The consumed power was calculated via Lissajous method using an additional capacitor of 0.1 μF connected in series between the mesh electrode and the ground as indicated in figure 1. Using these parameters, the power consumption varied between 0.15 W and 14 W.

2.2. Handling and treatment of Geobacillus stearothermophilus spore samples

The spore samples of *G. stearothermophilus* were prepared on stainless steel substrates with dimensions 30 mm × 6 mm × 0.5 mm (SIMICON GmbH, Munich, Germany). According to the specification given by the manufacturer, the number of spores on the sample was ∼1.5 × 10^6. For the treatment, a spore sample was placed on the top of a polyether ether ketone (PEEK) block. PEEK is highly resistant to electrical and thermal influences. A cylindrical quartz tube, made from fused silica, with an inner diameter of 32 mm and a height of 10 mm was placed around the sample. The SMD electrode was placed on the top of the quartz tube, with the grounded electrode down and the powered electrode matching exactly the position of the quartz tube as shown in figure 1. Thus, a volume between the spore sample and the SMD electrode was confined. No gas flow was applied to the confined volume; thus, the static, ambient air was used as background gas for all plasma treatments in this study. After the plasma treatment, the spore sample was put into a sealable tube with 5 ml of Ampuwa® (Fresenius Kabi Deutschland GmbH, sterile and pyrogen-free water) and vortexed for 30 s. The tube was then ultrasonically-bathed for 20 min and vortexed again for 30 s. Dilution series were made to evaluate the sporicidal effect by the plasma treatment. 100 μl of each suspension was inoculated onto a tryptic soy agar (TSA) plate. After the inoculation, the agar plates were incubated at 55.5 °C for at least 16 h. For each experimental parameter, three spore samples were treated. The experiments were repeated at least three times for all treatment conditions. Untreated samples were also processed in the described way in order to confirm the number of spores on the samples and the recovery rate by this method.
2.3. Measurement of temperature, UV power and humidity

The temperature was measured at the mesh electrode and at the substrate surface after 5 min of plasma generation using a type K thermocouple with a tip diameter of $\sim 0.6$ mm. The power consumption was varied between 0.5 W and 14 W. The room temperature was constant at 21 °C. Figure 4 shows the temperature increased with different power consumptions. The temperature difference increased linearly with increasing power consumption, with a maximum of 25 °C at the SMD electrode and 5 °C at the substrate at 14 W. Since \textit{G. stearothermophilus} spores are thermophile microorganisms and proliferate preferably at temperatures above 50 °C, the contribution of heat generated by SMD plasma to the sporicidal effect was excluded in this study.

The UV power was measured applying different powers using a UV power meter (H8025, Hamamatsu Photonics, Hamamatsu, Japan) in the UVC-range ($190 - 280$ nm). The UV sensor was placed 10 mm from the SMD electrode, according to the position of the spore samples. The UV dose was calculated by integrating the measured UV power over 5 min. Figure 5 shows the UV dose as a function of power consumption. In the low-power range below 5 W, relatively low UV doses of a few $\mu$J cm$^{-2}$ were measured. As the power was raised to 14 W, the UV dose increased monotonically to $\sim 40 \mu$J cm$^{-2}$, with the maximum below 50 $\mu$J cm$^{-2}$ at 14 W. This is by several orders of magnitude below the lethal dose for the inactivation of microorganisms (tens of mJ cm$^{-2}$) [43–45]. Therefore, UV radiation does not play a role for the sporicidal effect in this study.

The humidity was measured using a hygrometer (GFTB 100, Greisinger electronic GmbH, Regenstauf, Germany). In this study, we treated the spore samples under three different humidity conditions, referred to as condition A, condition B and condition C, with humidities of $5.5 \pm 0.5$ g m$^{-3}$, $10.4 \pm 0.6$ g m$^{-3}$ and $17.9 \pm 0.6$ g m$^{-3}$, respectively, where the error represents the respective standard deviation. In order to obtain the relatively high humidity of condition C, 40 $\mu$l of tap water was spread on the PEEK block using a cell spreader. For all the experiments,

![Figure 4. Temperature increase at the SMD electrode and the sample substrate after 5 min of plasma generation.](image)
the ambient temperature was 21 ± 1 °C and the pressure 960 ± 10 hPa. The humidity was measured just before and immediately after plasma treatment. No appreciable change of humidity was observed, and it was therefore assumed that the humidity was constant during plasma treatment.

2.4. Ozone measurement

The concentration of ozone was measured by optical absorption spectroscopy at the wavelength of 254 nm as described by Shimizu et al [43] using a mercury-argon lamp (Avalight-CAL, Avantes BV, Apeldoorn, Netherlands) and a spectrometer (AvaSpec-2048, Avantes BV). The ozone measurement was performed in absence of spore samples at a distance of 0.5 mm from the PEEK block, as illustrated in figure 1. Each measurement was repeated three times and mean values were calculated.

3. Results

The number of recovered colony forming units (CFUs) of *G. stearothermophilus* after 5 min of SMD plasma treatment under different plasma conditions at different humidities is shown in figure 6. The number of survived CFUs was illustrated in box plots where the lower and upper edges of each box indicate the first and the third quartiles, respectively, and the bar inside each box, the median. The whiskers indicate 1.5 times of the interquartile range (IQR) and cover 99.3% of the measurement data, including the box. The detection limit for the CFUs was 25.

Under condition A, almost no sporicidal effect was observed in the investigated power range between 0.5 W and 14 W. The reduction of spores was near zero, with the maximum below 0.5 log. Under condition B, almost no spore reduction was observed in the lower power range below 3 W. With increasing power consumption (up to 5 W), spore reduction increased monotonically. As the power consumption increased, the spore reduction decreased and
dropped to almost zero at 10.5 W and higher. Thus, a peak in the sporicidal effect was observed at the power consumption level of 5 W, with the maximum spore reduction of approximately 2.5 log. Under condition C, the spore reduction increased monotonically as the power consumption was increased. Spore reduction of more than 3 log was observed at the applied power of 14 W. Under conditions B and C, the fluctuation in the recovered number of CFUs was large, with a range of up to 2.5 log. The plasma and gas conditions are assumed to be constant and uniform during plasma treatment. The fluctuation can possibly be explained by the surface conditions of the spore samples, which were manufactured by drying a definite amount of a spore suspension on the substrates. Klämpf [56] showed by means of SEM images that cell debris and salt crystals from the manufacturing procedure were randomly distributed over the sample surface. In addition, the spore cells, too, were randomly distributed over the sample surface and partly formed multiple layers. The cell debris, salt crystals and multiple layers of spore cells could have shielded the underlying spores from the sporicidal agents and from the SMD plasma. This shielding effect was individual for each spore sample and could have varied greatly among the samples, resulting in a large fluctuation of the recovery after SMD plasma treatment. Note that the standard deviation of the recovery from the untreated spore samples (negative control) was 0.3 log.

To investigate the kinetics of spore reduction, the exposure duration of the spore samples to the SMD plasma was increased from 1 to 5 min under all three humidity conditions. The power consumption was fixed at 5 W, where the maximum sporicidal efficacy under condition B was found. Under condition A, almost no sporicidal effect was observed with a reduction efficacy below 0.5 log even after 10 min of SMD plasma treatment (data not shown). At the power consumption of 5 W, the spore reduction was similar under conditions B and C after
5 min of SMD plasma treatment (2–2.5 log). As shown in figure 7, the survival curves exhibited a linear increase of sporicidal efficacy on the logarithmic scale as treatment duration increased.

In order to understand sporicidal mechanisms, it is important to investigate the chemical components in the volume where the spore samples were treated. As an indicator for the long-lived reactive species, the concentration of ozone was measured. The evolutions of the ozone concentration under condition A are shown in figure 8. The error bars represent the standard deviation from three independent measurements. In the low-power range below 0.5 W, the ozone concentration saturated after \( \sim 50 \) s. The saturation concentration increased with increasing power consumption and reached its maximum of around 2500 ppm at 0.5 W. As power consumption was increased further, the ozone concentration started to deplete tens of seconds after plasma ignition. The depletion of ozone accelerated as the power consumption
was increased. While decreasing only by 20% after 300 s at 1.5 W, the ozone concentration dropped to zero within 300, 70, 25 and 10 s at 3, 5, 8 and 14 W of power consumption, respectively. The production rate of ozone, determined by the slope of the time evolution curve in the first seconds after the plasma ignition, increased with increasing power consumption. These observations are qualitatively in accordance with the results presented by Shimizu et al. [43].

Based on the obtained time evolution data, the average ozone concentrations over 5 min were calculated. Figure 9 shows the average ozone concentration at different power consumptions and under different humidity conditions. The shape of the average ozone curves was similar under all three humidity conditions, showing a maximum of between 0.5 and 1.5 W of power consumption. At high-power consumptions of 8 W and higher, the average ozone concentration remained below 50 ppm under all humidity conditions. The average ozone concentration depended strongly on the humidity. With higher humidity (condition C), the maximum average ozone concentration was lower and below 500 ppm. In contrast, ozone concentrations of over 2000 ppm were measured at 0.5 W with lower humidity (condition A).

4. Discussion

The thermophile properties of *Geobacillus stearothermophilus* and the low heat dissipation at the SMD electrode eliminate the possibility of spore inactivation by heat. The measured UV power was several orders of magnitude below the lethal dose for microorganisms of \( \sim 10 \) mJ cm\(^{-2}\). Due to the distance of approximately 10 mm from the spore samples to the SMD electrode and the fact that the transport of the reactive species was mainly by diffusion with the characteristic transport time of seconds, the direct contribution of short-lived reactive species, especially electrons and ions, to the sporicidal effect was ruled out as well [46]. It was assumed that long-lived reactive species were primarily responsible for the inactivation of spores in this study. The single-slope linear decrease of the survival curves suggests that only one main
mechanism was involved in the inactivation of spores by the SMD plasma under the investigated conditions.

Ozone is one of the possible long-lived sporidical agents and has been indicated as a major bactericidal agent from SMD plasma by recent studies [43, 45]. Ozone is produced mostly by the reaction
\[ \text{O} + \text{O}_2 + \text{M} \rightarrow \text{O}_3 + \text{M}, \] (1)
where M represents a third body. The production of ozone depends substantially on the availability of atomic oxygen produced by the electron dissociation of oxygen molecules:
\[ \text{e} + \text{O}_2 \rightarrow \text{O} + \text{O} + \text{e}. \] (2)

Dorai et al showed the average densities of both atomic oxygen and hydroxyl radicals produced by corona plasma as functions of humidity [47]. Water molecules are dissociated to hydroxyl radicals by electrons:
\[ \text{e} + \text{H}_2\text{O} \rightarrow \text{OH} + \text{H} + \text{e}. \] (3)

Generally, larger amounts of both atomic oxygen and hydroxyl radicals are produced if the electron yield increases due to higher power consumption. At a given power consumption, reactions (2) and (3) are in competition. With increasing humidity, reaction (3) is promoted and the concentration of OH increases while the production of O and ozone decreases. This model is qualitatively in good agreement with the results from the ozone measurements performed in this study.

The average ozone concentration was inversely proportional to humidity, while the sporidical efficacy generally increased with the humidity. At high ozone concentrations under condition A, almost no sporidical effect was observed. Under condition B, a maximum of sporidical efficacy was observed at 5 W of power consumption, where ozone was mostly quenched in less than 60 s. Under condition C, the lowest ozone concentrations were measured and the average ozone concentration decreased with higher power consumption. The highest spore reduction was observed under this condition, and the sporidical efficacy increased monotonically with power consumption. These observations indicate that ozone did not contribute much to the inactivation of *G. stearothermophilus* spores by the SMD plasma in this study.

At the low humidity of 5.5 ± 0.5 g m\(^{-3}\) under condition A, almost no sporidical effect was observed, even after 10 min of SMD plasma treatment. At the high humidity of 17.9 ± 0.6 g m\(^{-3}\) under condition C, the highest sporidical effect was obtained, with a spore reduction of up to 3–3.5 log. These results indicate that water vapor was necessary for the inactivation of *G. stearothermophilus* by the SMD plasma. Water molecules can be dissociated by electrons and produce OH radicals according to reaction (3) and/or produce H atoms and hydroperoxyl radical (HO\(_2\)) via different cascades of reactions [41, 46, 47]. Even though OH, H and HO\(_2\) are short-lived species, their contribution to the sporidical effect cannot be ruled out at high humidity since they can be produced by the reaction of the long-lived species such as ozone with the water molecules in vicinity of the spore sample. As mentioned earlier, Hähnel et al suggested that OH radicals play an important role in the inactivation of spores by the humid-air plasma produced by DBD [37]. Moreover, long-lived water-related species such as hydrogen peroxide produced from the reactions contributed to the inactivation of spores. H\(_2\)O\(_2\) is one of the most well-known sporidical agents and widely used in the sterilization processes [48–50].
addition, reactive nitrogen species, e.g. NO2, N2O5 and HNO3 can contribute to the sporicidal effect as well, as suggested by Schnabel et al [51].

At the humidity of 10.4 ± 0.6 g m⁻³ under condition B, a maximum of spore reduction of ∼2.5 log was observed at the power consumption of 5 W. Higher power consumption depleted the responsible species for the sporicidal effect. It is still under discussion what species were the mainly responsible agents. Further detailed investigations, especially on the long-lived reactive species apart from ozone, are necessary. Under condition C, the reduction of spores increased monotonically with power consumption. We observed that there was condensation on the spore sample surface when the power consumption exceeded 1 W. It is possible that the spore samples have been affected by the plasma-activated water (PAW) [44, 52] containing a variety of reactive species, including short-lived species, e.g. HO2. Jung et al reported that OH radicals were formed during spore inactivation by aqueous ozone and UV that enhanced spore reduction by ∼33% [53]. The acidification of the PAW can enhance the sporicidal effect [54]. Since the sporicidal efficacy between 0.5 W to 5 W was similar under conditions B and C, it is possible that the power-dependent spore reduction at high humidity under condition C was the superposition of the maximum observed at 5 W under condition B with additional effects from the PAW. Further investigation is required to find out the main inactivation mechanism, including the contribution of PAW by the SMD plasma.

5. Conclusion

With the rapid development of medical and hygiene standards of the recent decades, sterilization and disinfection have become increasingly important. The conventionally used sterilization methods either apply toxic chemicals or produce heat and often require expensive systems. Here the SMD offers an attractive alternative with a wide range of applications in the fields of medicine, hygiene and the food industry. Previous studies have demonstrated the beneficial properties of the SMD, especially the inactivation of bacteria, fungi, yeast, biofilms, viruses and bacterial spores. In this study, the highly resistant bacterial endospores of Geobacillus stearothermophilus were exposed to the SMD plasma. In order to optimize the inactivation of spores, the parameters for plasma generation and humidity were varied over a wide range. The contribution of heat and UV to the sporicidal effect was negligibly small. The involvement of the short-lived species produced in the SMD plasma was excluded, since the spore samples were placed at a distance of 10 mm from the electrode. The long-lived reactive species were considered responsible for the inactivation of spores and the concentration of ozone was measured as an indicator.

We observed more than 3 log of spore reduction in 5 min depending on the humidity and power consumption. The sporicidal effect depended substantially on the humidity. Almost no sporicidal effect was observed at the low humidity of 5.5 ± 0.5 g m⁻³, while the highest effect was observed at the highest humidity of 17.9 ± 0.6 g m⁻³. This indicates that water-related species, e.g. H2O2, could be the major sporicidal agents. At the humidity of 10.4 ± 0.6 g m⁻³, a maximum of sporicidal efficacy was found at 5 W of power consumption. The contribution of ozone to the sporicidal effect was negligibly low because no correlation between the measured ozone concentration and the reduction of spores was found. It cannot be excluded that ozone contributed to chemical reactions that produced water-related sporicidal species such as OH and H2O2. At high humidity, condensation of water was observed on the spore sample surface.
during plasma treatment at power consumptions above 1 W. The condensed PAW can interact with the reactive species and contain both short-lived and long-lived reactive species. It is possible that this PAW contributed to the inactivation of the spores. Further investigation is necessary in order to specify the mechanisms of spore inactivation and to estimate the contribution of the PAW.

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