

Suitability of thermal plasmas for large-area bacteria inactivation on temperature-sensitive surfaces – first results with *Geobacillus stearothermophilus* spores

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Abstract. The application of thermal plasma for large-area bacteria inactivation on temperature-sensitive surfaces is not a common one. Nonetheless, there are thermal plasma generators which offer a high sheath homogeneity and have proven to be suitable for treatment of thermally sensitive materials in the past. To investigate the suitability of such plasmas, agar dishes plated with endospores of *Geobacillus stearothermophilus* have been treated with a long arc plasma generator called LARGE. The achieved results have been compared with a commercially available non-thermal plasma generator. A significant inactivation of the endospores could be observed only after 60 s of treatment with the thermal plasma source. This was not possible with the non-thermal generator. Moreover, no temperature damage or increase of the specimen could be detected. An attempt to determine the main agents responsible for the microbicidal effects have been made – the influence of plasma gas composition, discharge current and treatment time has been investigated. Significant improvements in the disinfection rates after adding small amounts of nitrogen to the plasma gas could be observed. A first discussion regarding the suitability of thermal plasmas for bacteria inactivation has been given.

1. Introduction

Treatment with non-thermal plasmas is by now a well-established method for bacteria inactivation in medical and biological research and has been investigated by many researchers [1, 2, 3]. As stated in the literature various plasma agents and properties are responsible for the sterilisation effect. In general, the main agents are: temperature, UV radiation, reactive nitrogen and oxygen species (short RNS and ROS, respectively) and short-lived charged particles [4, 5]. Although the sterilizing agents can be defined, many researchers state that the interaction effects between plasma and bacteria are not fully understood [2, 3, 6, 7]. This may be due to insufficient or missing plasma diagnostics. A major challenge thereby is to quantify the reactive and therefore very short-lived species, whose concentration is often very low and decays rapidly in effluent plasma jet under atmospheric pressure [8, 9].

The application field of such plasmas can be manifold. They are often used as an alternative to traditional sterilization methods of stents, implants, scalpels or other surgical instruments [7, 10], for bacteria inactivation [11, 12] or wound healing [13, 14]. Moreover, investigations in the fields of water and air decontamination [15, 16], treatment of heat- and humidity-sensitive food as well as inactivation of chemical and biological warfare agents [17, 18] have been performed by various



researcher groups. There are even some commercially available CE-certified atmospheric pressure cold plasma sources for medical treatments [19, 9].

Most of the investigated non-thermal plasmas have a jet- or pen-shaped geometry, which allows a point-like surface treatment with a diameter of several mm². Nonetheless, concerning the application fields, a need for large-area surface decontamination can be easily recognized. Efforts to create a large-area surface sterilisation by upscaling a non-thermal discharge or stringing together several cold plasma jets had already been undertaken by various researchers [20, 21, 22]. The stability and plasma sheath homogeneity is one of the main concerns of such systems [1, 21].

Attempts to realize atmospheric pressure large-area plasma generators with high plasma sheath homogeneity have been successfully undertaken by some groups [23, 24]. These designs were based on thermal plasmas rather than non-thermal ones. Farrar et al. studied even the applicability of their thermal plasma generator for large-area surface sterilisation [23]. Investigated application were the treatment of runways, roads or buildings, all of those non temperature-sensitive surfaces. No further literature which described the application of thermal plasmas for bacteria inactivation on temperature-sensitive surfaces could be found.

One thermal plasma generator with high sheath homogeneity designed for large-area treatments, which has also shown good suitability for large-scale surface treatment of temperature-sensitive substrates in previous works [24, 25, 26], has been developed at the LPT and is called LARGE. Within this work, the suitability of thermal plasmas (produced by LARGE) for large-area bacteria inactivation on temperature-sensitive surfaces has been investigated.

2. Approach

Within this section the function principle of the used plasma sources has been described. Additionally, the choice of bacterium has been explained as it is of great importance for following results.

2.1. LARGE – Long ARc plasma GEnerator

LARGE, a long arc plasma generator, is a linear DC-plasma source with an up to 450 mm long electrical arc discharge, where the plasma gas is fed perpendicularly to the arc. A high temporal stability of its operating parameters, and hence of the plasma sheath, can be achieved, since the arc length is defined by the distance between the electrodes and the arc is being stabilized by water-cooled cascades and a magnetic barrier stretching along the whole arc length, as shown in figure 1. Insulating plates are placed between each element of the cascade to prevent a current flow through one of them, and thus force the arc to burn between the main electrodes. The cascaded layout facilitates also an easier arc ignition. The Lorentz force created by the two permanent magnets on the other hand, prevents an arc deflection caused by the lateral plasma carrier gas flow, figure 2. Such a gas injection allows the generation of thermal plasmas with different (also aggressive or oxidative) gases, such as Ar, N₂, O₂, compressed air, H₂, He, CH₄ or CO₂ [26].

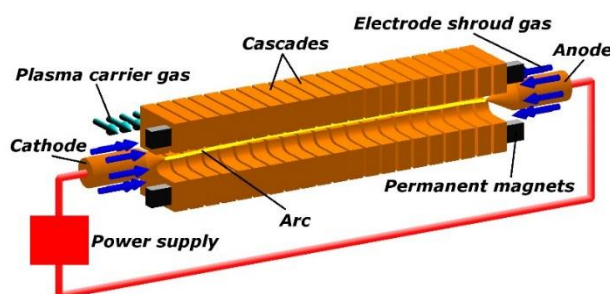


Figure 1. Schematic layout of the thermal plasma generator LARGE.

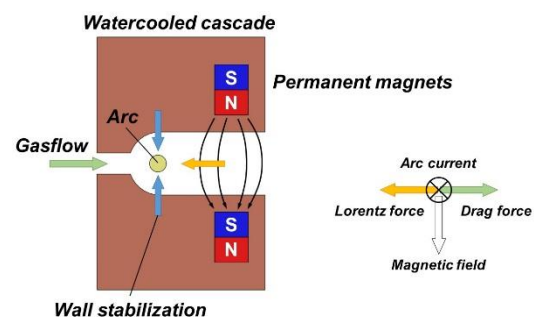


Figure 2. Cross-section of a LARGE cascade describing the arc stabilization principle

Depending on the gas type and chosen process parameters, temperatures between 100 °C and 4500 °C in the effluent plasma sheath at generator exit can be achieved. According to that, all main agents responsible for plasma enhanced decontamination can be generated and adjusted within a relatively wide range. Additionally, due to high arc stability, a large-area temporarily and spatially homogenous plasma sheath can be created. Because of its characteristics the LARGE seems to be suitable for large-area decontamination of temperature-sensitive surfaces and therefore should be used within this work.

2.2. Non-thermal plasma generator for comparison reasons - Relyon Plasma PB3

As a reference point the commercially available non-thermal plasma system, PB3 plasma generator and a PS2000 power supply, both produced by Relyon Plasma GmbH, Regensburg, Germany, has been used. According to the manufacturer this generator can be used for surface sterilization in industrial applications, for ex. packaging [27]. The plasma generator PB3 has a more conventional construction with a finger-shaped inner electrode and a nozzle acting as a cathode, as presented in figure 3. A unipolar pulsed high voltage (a few kV) arc discharge is used to generate a plasma jet. To prevent a pre-mature electrode wear, a vortex flow of the plasma carrier gas inside the nozzle is introduced and so the arc rotates inside the discharge chamber at a high frequency. Similarly to the LARGE, different working gases can be used, most commonly compressed air, N₂, CO₂ or N₂/H₂-mixtures. Furthermore, the plasma temperature can be adjusted by varying the nozzle length. The system does not require any additional cooling.

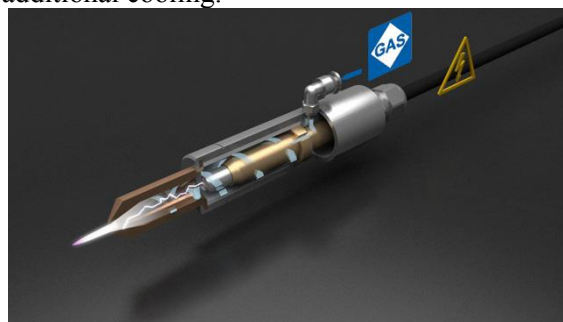


Figure 3. Schematic layout of the non-thermal plasma generator PB3 used for comparison reasons.

2.3. Bacteria choice - *Geobacillus stearothermophilus* ATCC 7953

The choice of bacterium used to determine the sterilisation effects of a plasma appears to be of particular importance. As shown by Morris et al. [28] significant differences in inactivation rates can be observed between a vegetative bacterium and its endospore. The authors examined the effects of treatment with a cold atmospheric pressure plasma (direct and indirect treatment) on *Geobac. stearothermophilus* and *Bacillus cereus* in both forms, endospore and vegetative. *Geobac. stearothermophilus* is a spore-forming, gram-positive rod-shaped aerobic bacterium, whose optimum growth temperature is between 50 °C and 65 °C [29]. During investigations the treatment time has been varied between 10 s and 30 min. No further information about the used plasma gas and other process parameters has been given. The results show that after treatment with the cold plasma both vegetative forms of *Geobac. stearothermophilus* and *Bacillus cereus*, as well as the *Bacillus cereus* spores regardless of whether plated on agar dishes or on glass specimen could be effectively inactivated. The endospores of *Geobac. stearothermophilus* however, could not be significantly inactivated by any sort of plasma treatment, neither indirect nor direct one.

According to Yardimci and Setlow [30] spores of *Bacillus* strain and their close relatives (*Bac. atrophaeus*, *Bac. cereus*, *Bac. subtilis* and *Geobac. stearothermophilus*) possess a significantly higher resistance against plasma treatment than the vegetative forms of these strains or other bacteria. This applies under the assumption of identical treatment conditions, even if different plasma types were used for the experiments. Also other comparative studies conclude that the endospores of *Geobac. stearothermophilus* are among the species with the greatest resistance to a plasma treatment and

therefore can be used as a suitable biological indicator for the assessment of the microbicidal effect of a plasma [31].

Hence, spores of *Geobac. stearothermophilus* appear to be particularly well suited to prove the suitability of thermal plasmas for large-area bacteria inactivation on temperature-sensitive surfaces.

3. Experimental setup

To show the suitability of thermal plasmas for bacteria inactivation on temperature-sensitive surfaces a simple two-step analysis method (screening and quantification) has been applied. At first, tests with a dense bacteria layer have been conducted to screen the parameters and determine the main influencing factors. Therefor 50 µl of an undiluted base suspension containing 2.2×10^6 CFU/ml (CFU – colony forming unit) of *Geobac. stearothermophilus* ATCC 7953 spores have been plated onto tryptone soya agar (TSA) dishes and incubated for 24h at 56 °C after treatment. The main focus was to determine the influence of gas composition, treatment time and distance as well as input power, i.e. arc current or pulse frequency depending on the used plasma system.

After the determination of the influencing factors, disinfection rates have been determined by cell counting to quantify the plasma effects. To do so a 1:121 dilution of the base suspension (2.2×10^6 CFU/ml) has been used. Again, 50 µl of this dilution, which contain an average of 909 CFU, have been distributed onto TSA plates and incubated under similar conditions after the plasma treatment. The treatment distance for these investigations has been kept constant at 60 mm. Other parameters, set based on the results with dense bacteria layers, were examined and fine-adjusted if needed. Moreover, for statistical reasons, three samples have been analysed for each parameter set. The detailed LARGE parameters are presented in table 1.

Table 1. Parameters set for the investigations with thermal plasma generator LARGE.

	Process parameter:	Value:
constant	Arc length	150 mm
	Electrode shroud gas	Ar
	Shroud gas flow rate	2.5 l/min
	Total gas flow rate	35 l/min
	Treatment distance	60 mm
variable	Base plasma carrier gas type	Ar
	Additional plasma carrier gases	N ₂ , O ₂
	Additional gas flow rate	5 l/min (14 Vol.-%), 10 l/min (28 Vol.-%)
	Arc current	40 A, 50 A
	Treatment time	15 s, 30 s, 45 s, 60 s, 120 s

The results achieved with the LARGE have been compared with a commercially available non-thermal plasma generator (Relyon Plasma PB3). All experiments with both plasma generators have been performed stationarily, i.e. without moving the dishes or the plasma generator, as shown in figure 4 and figure 5.

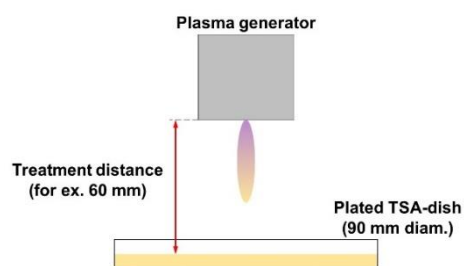


Figure 4. Schematic diagram of the testing conditions. Experiments have been performed stationary.

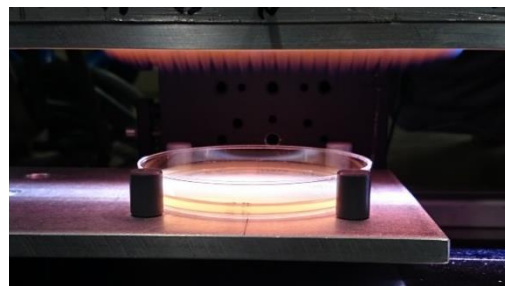


Figure 5. A plated TSA-dish is being treated with the thermal plasma generator LARGE. No thermal degradation could be observed.

4. Results

The results presented within the following section concentrate primarily on the results achieved with thermal plasmas, rather than non-thermal one.

4.1. Screening – determining the main influencing factors

First sterilization experiments with a dense microbial biofilm of *Geobac. endospores* showed that a significant amount of spores could be killed after just 60 s of treatment with the thermal plasma generator LARGE (figure 6). In comparison, a significant deactivation of the endospores could not be observed even after a four times longer treatment of 240 s with the non-thermal plasma generator PB3. Moreover, after such a long treatment with the cold plasma jet a thermal degradation of the agar dish could be observed. As shown in figure 7, a significant bacteria inactivation after a treatment with the PB3 generator could only be observed after a pre-incubation of plated dishes (6 h at 56 °C). Hence, only the vegetative form of *Geobac. stearothermophilus* could be effectively inactivated with the cold plasma, what coincides with the results obtained by Morris et al. [29]. For such testing conditions (vegetative form, static treatment), best results with the PB3 could be achieved in a treatment distance of 60 mm, as shorter distances caused a thermal damage of the substrates and longer ones lessened the effect. According to that, for comparison reasons, the distance of 60 mm has been kept unchanged for LARGE experiments.

The experiments showed also that plasma gas composition plays a key role in sterilization processes regardless of the used plasma generator type. In the case of the LARGE significant improvements can be achieved when small amounts of N_2 or O_2 are added to the plasma carrier gas (Ar). An arc current increase (from 40 A to 50 A) also improved the disinfection effects. The positive effect was however much lesser in comparison to gas composition change. Furthermore, compared to the non-thermal plasma jet, thermal degradation of the agar could not be observed even after 120 s treatments with the LARGE. The average gas temperature measured in the treatment distance of 60 mm was 23.6 °C (PB3 in comparison: 84.1 °C). Furthermore, as one expected, better inactivation rates (to an extent) could be achieved by elongating the treatment time (see section 4.2).

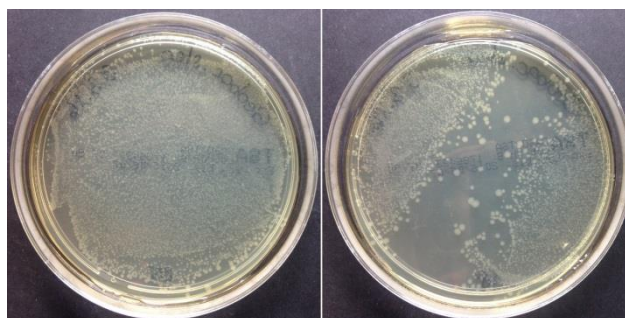


Figure 6. *Geobac. stearothermophilus* ATCC 7953 spores before (left) and after (right) 60 s of treatment with the LARGE. Parameters: plasma carrier gas flow: 20 l/min Ar + 10 l/min N₂, electrode shroud gas flow: 1.5 l/min, arc current: 50 A, treatment distance: 60 mm



Figure 7. Comparison of results after 60 s of treatment. Left: non-thermal plasma (PB3), dish pre-incubated after plating for 6h at 56°C. Right: thermal plasma (LARGE), direct treatment of endospores

In summary it can be said, that the thermal plasma (produced by the LARGE) seems to be suitable for a large-area bacteria inactivation. Moreover, the key parameters in this application field are the plasma gas composition followed by the treatment time and the arc current.

4.2. Quantification – determining the disinfection rates

To quantify the antimicrobial impact of the thermal LARGE-plasma, a dilution of the base suspension containing an average of 909 CFU has been used. As previously conducted tests show, plasma carrier gas composition and treatment time have the highest impact on bacteria inactivation. Moreover a significant improvement could be observed when small amounts of nitrogen or oxygen were added. Accordingly to that, quantification tests with pure argon as plasma carrier gas have been skipped. A N₂-O₂ mixture has not been investigated as it would complicate the differentiation of the plasma agents responsible for the sterilization effect. The determined disinfection rates for different gas compositions have been shown in figure 9. Best results for a constant treatment time of 60 s could be achieved for nitrogen (5 l/min = 14 Vol.-%). Therefore, the treatment time study has been conducted for an Ar-N₂ (14 Vol.-%) plasma gas mixture and an arc current of 50 A. Other parameters have been set according to table 1. As shown in figure 8, a significant degradation of CFUs could be observed just after 30 s of treatment. Further extension of the plasma exposition time increases the impact only marginally. Noteworthy, as stated already before, no thermal degradation could be observed during these tests. In both figures the untreated samples have been marked as reference (Ref).

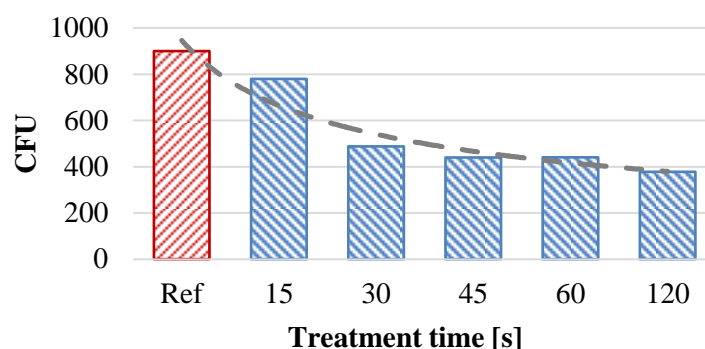


Figure 8. Bacteria inactivation rate after LARGE treatment as a function of treatment time. Parameters used: Ar-N₂ mixture (14 Vol.-%, i.e. 25 l/min + 5 l/min) and arc current of 50 A.

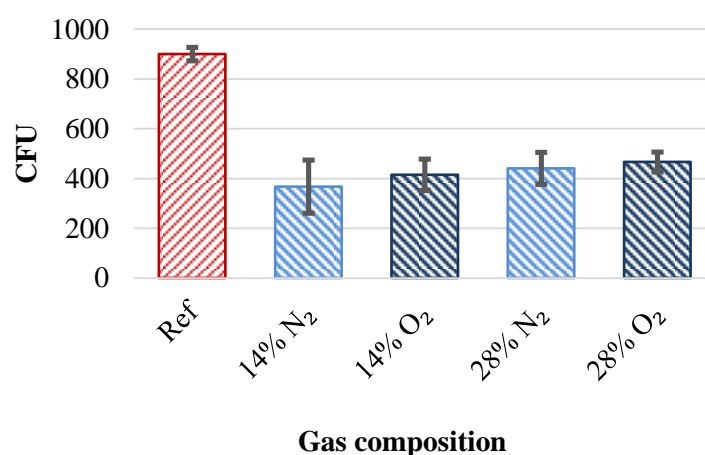


Figure 9. Influence of the plasma gas composition on the bacteria inactivation using thermal LARGE-plasma. Parameters used: arc current of 50 A and treatment time of 60 s.

5. Discussion

As could be shown, whether a thermal or a non-thermal generator is being used, the choice of plasma carrier gas seems to be important for the microbicidal effect of a plasma. Nonetheless, only with thermal plasma a significant inactivation of *Geobac. stearothermophilus* spores could be achieved. In addition to that, the best results could be achieved for an Ar-N₂ (14 Vol.-%) mixture. A possible reason for that could be an increased generation of RNS (and maybe ROS) due to secondary reactions with ambient air. A change in the pH-value of the treated TSA-dishes could also be a conceivable explanation. Interestingly, a similar addition of oxygen or an increased nitrogen concentration seems to provide slightly worse results. At the moment, no unambiguous explanation can be provided for this effect. Further investigation of different gas compositions coupled with spectroscopic measurements may help to understand this effect.

Moreover, a slight increase in arc current (from 40 A to 50 A) also improved the effects, the positive effect was however much lesser in comparison to gas composition change. As the intensity of UV radiation is expected to increase with rising current, the main killing agents seem to be RNS and ROS. Thermal degradation of the agar, which could be partially observed after treatments with the non-thermal plasma generator, could not be seen after LARGE treatments. Although the estimated energy densities being in a similar range, significantly lower gas temperatures when using the LARGE could be measured (LARGE: 23.6 °C; PB3: 84.1 °C) in the treatment distance of 60 mm. Thus, heat seems not to be responsible for the killing.

All in all, the reduction in disinfection rates of *Geobac.* endospores amount only to about 50 %, but keeping in mind the test conditions (static treatment, 60 mm distance), there is no doubt about that far

better results are possible if the plasma generator is being moved or the sample is directly treated with plasma.

6. Conclusion and Outlook

As the investigation results show, thermal plasmas can be applied for large-area bacteria inactivation on temperature-sensitive surfaces. Due to the wide range of possible parameter adjustments, the long arc plasma generator LARGE showed good potential for sterilization applications. Furthermore, a direct sterilization of *Geobac. stearothermophilus* endospores was possible, which was not the case for the non-thermal plasma generator. A significant reduction of CFUs just after 30 s treatment time could be achieved – further improvements possible with detailed parameter adjustments (for ex. treatment distance) and other testing conditions (non-static treatment). Most importantly, no thermal load of the treated surfaces could be determined. The main killing agents, for now, seem to be the reactive nitrogen and oxygen species. Nevertheless, as discussed in chapter 5, there are still some open questions regarding the observed effects. The exact plasma agents produced by the thermal plasma generator and responsible for bacteria inactivation remain to be explored in future work.

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