

Plasma sterilization. Methods and mechanisms*

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Abstract: Utilizing a plasma to achieve sterilization is a possible alternative to conventional sterilization means as far as sterilization of heat-sensitive materials and innocuity of sterilizing agents are concerned. A major issue of plasma sterilization is the respective roles of ultraviolet (UV) photons and reactive species such as atomic and molecular radicals. At reduced gas pressure (≤ 10 torr) and in mixtures containing oxygen, the UV photons dominate the inactivation process, with a significant contribution of oxygen atoms as an erosion agent. Actually, as erosion of the spore progresses, the number of UV photons successfully interacting with the genetic material increases. The different physicochemical processes at play during plasma sterilization are identified and analyzed, based on the specific characteristics of the spore survival curves.

INTRODUCTION

Sterilization is an act or process, physical or chemical, that destroys or eliminates all forms of life, especially microorganisms. Conventional sterilization techniques, such as those using autoclaves, ovens, and chemicals like ethylene oxide (EtO), rely on irreversible metabolic inactivation or on breakdown of vital structural components of the microorganism. Plasma sterilization operates differently because of its specific active agents, which are ultraviolet (UV) photons and radicals (atoms or assembly of atoms with unpaired electrons, therefore chemically reactive, e.g., O and OH, respectively). An advantage of the plasma method is the possibility, under appropriate conditions, of achieving such a process at relatively low temperatures (≤ 50 °C), preserving the integrity of polymer-based instruments, which cannot be subjected to autoclaves and ovens [1,2]. Furthermore, plasma sterilization is safe, both for the operator and the patient, in contrast to EtO.

The UV photons are expected to be more strongly (if not totally) reabsorbed by the ambient gas at atmospheric pressure than at reduced pressure (≤ 10 torr). This led us to consider two types of sterilizers: atmospheric- and reduced-pressure systems [1,2]. The respective roles of UV photons and radicals at reduced pressure are already well understood [1–4], which is not the case yet at atmospheric

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pressure [5,6]. Our presentation focuses on reduced-pressure operation and is based on the analysis of the specific characteristics of the microorganism survival curves resulting from plasma sterilization.

In both systems, the devices to be sterilized can be arranged to be either in contact with the discharge or with its flowing afterglow. Figure 1 shows a typical flowing-afterglow sterilizer.

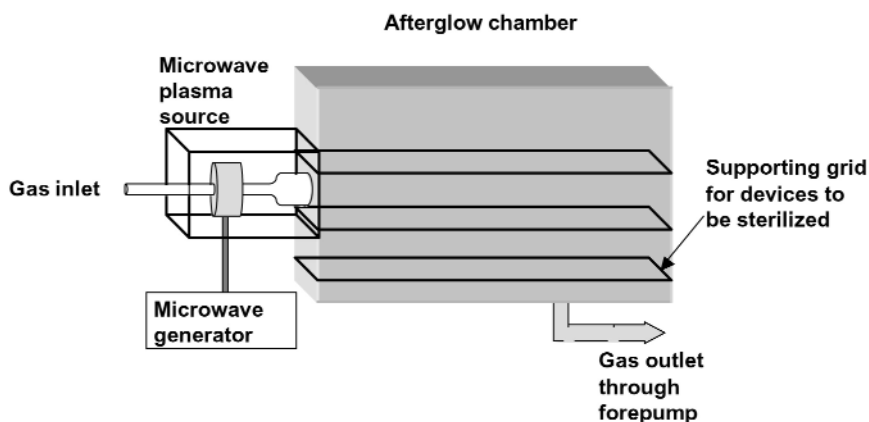


Fig. 1 Flowing-afterglow 50-L sterilizer developed at Université de Montréal. It is supplied from a 2450-MHz surface-wave discharge operated at the 100–150 W power level in a 6 mm i.d. Pyrex tube. Pressure is in the 1–10 torr range with a gas flow rate typically between 0.5 and 2 standard L/min. The Petri dish is located at 10–15 cm from the afterglow chamber entrance.

SURVIVAL CURVES

We have recently shown [1,2] that the physicochemical processes taking place during a plasma sterilization cycle could be identified by analyzing *survival curves*, a plot of the logarithm of the number of surviving microorganisms as a function of exposure time to the plasma. Such curves are usually obtained by inactivating bacterial spores, an extremely resistant form of microorganisms classically employed for testing and checking sterilizers.

Exposing microorganisms of a given type to conventional sterilization systems such as autoclaves, ovens and EtO generally yield survival curves with a unique straight line: the whole inactivation process up to sterilization is an exponential function of time [2,7]. In contrast, exposure to plasma (be it direct or in its afterglow) provides survival plots with 2 or 3 different linear segments, as shown in Fig. 2. This implies that the number of surviving microorganisms decreases, to a first approximation, as an exponential function of time also, but with different time constants, i.e., different kinetics.

Spore inactivation in Fig. 2 stems from the flowing afterglow of a microwave-sustained discharge in pure argon and in a mixture of 5 % O₂–95 % Ar [8]. The pure argon discharge yields, within a 40 min cycle, a 2-segment plot with a remaining large number of survivors, while the addition of O₂ leads, over the same time interval, to 3 linear segments and sterilization. We believe that the pure argon survival curve reflects inactivation by UV photons only since no radicals from the gaseous phase are available. In contrast, when adding O₂, which (partially) dissociates to yield O, then both UV and O atoms contribute to the inactivation of the spores.

To characterize the slope of each segment, to which we refer to as an *inactivation phase*, we use the time D required to decrease a given population of spores by a factor of 10 (90 % reduction). Standard procedures for qualifying and testing sterilizers require the inactivation of 10⁶ microorgan-

isms. Sterility is indicated in the survival plot by a fraction smaller than unity, the inverse of the number of independent experiments with no spores detected by culture on solid media. This number is an estimate of the probability of finding no survivors. In Figs. 2 and 3, for example, sterility is observed at 40 min and it is plotted as 1/6, because no spores were found over the 6 independent experiments performed. The error bars in the figures indicate the corresponding standard deviation. As a rule, phases 1 and 3 have approximately the same D value and these are much shorter than the D_2 value.

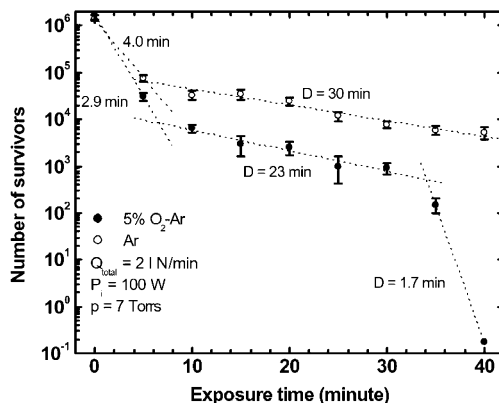


Fig. 2 Survival curves of *B. subtilis* spores exposed to a flowing afterglow. Adding O_2 to the argon in the discharge leads to a complete inactivation of the spores within 40 min. [8].

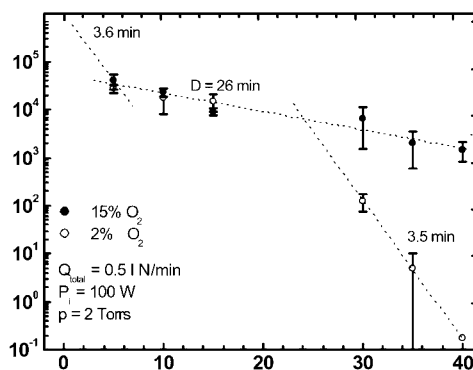


Fig. 3 Survival curves of *B. subtilis* spores subjected to a flowing afterglow in two O_2-N_2 mixtures: the 15 % O_2 mixture yields the highest oxygen-atom concentration, while the 2 % O_2 mixture maximizes the NO_β UV emission at this pressure and gas flow rate [8].

SYNERGISTIC EFFECT BETWEEN O ATOMS AND UV PHOTONS

Figure 3 compares the inactivation rate obtained in the afterglow of two different O_2-N_2 mixtures. The action of O_2 on the spores is assumed to result from oxygen atoms, while the relatively strong UV emission stems from excited $NO(B)$ molecules, formed in the afterglow as a result of collisions between N and O atoms. The range of the NO_β band (250–320 nm) actually includes the 220–270 nm spectral interval over which DNA destruction is the most efficient [3,9]. The aim of the experiment is to determine whether the UV intensity or the concentration of the radical O should be maximized to shorten

sterilization time [8]. The oxygen atom concentration [O], determined by a titration method [10], is found to first increase when increasing the O₂ percentage in the mixture and then reach a maximum value and saturate at O₂ percentages higher than 12 %. In contrast, the NO_β emission intensity goes through a maximum as a function of O₂ around 2 % O₂ in the mixture at 2 torr and 1 standard L/min. Figure 3 then clearly shows that the shortest sterilization time is obtained when the UV photon intensity is maximized. Considering both previous figures, we conclude that to achieve sterilization in less than an hour in our afterglow system: (i) oxygen atoms and UV photons are required (Fig. 2); (ii) the UV photon flux must be sufficiently intense (Fig. 3). As a result, one can speak of a synergistic effect between O atoms and UV photons.

BASIC MECHANISMS OF PLASMA STERILIZATION

Our interpretation of the survival curves assumes the three following elementary processes [1,2]:

- A) Destruction by *UV irradiation* of the genetic material of the microorganism; this is a statistical process requiring a sufficient number of lesions of the DNA strands.
- B) Erosion of the microorganism, atom by atom, through *intrinsic photodesorption*. Photon-induced desorption results from UV photons breaking chemical bonds in the microorganism material and leading to the formation of volatile compounds from atoms intrinsic to the microorganism. The volatile by-products of this nonequilibrium chemistry are small molecules (e.g., CO and CH_x) [11].
- C) Erosion of the microorganism, atom by atom, through *etching*. Etching stems from the adsorption of reactive species from the plasma (glow or afterglow) on the microorganism with which they subsequently undergo chemical reactions to form volatile compounds (spontaneous etching). The reactive species can be atomic and molecular radicals, for example, O and O₃, respectively, and excited molecules in a metastable state, for example, the ¹O₂ singlet state. This chemistry, which occurs under thermodynamic equilibrium conditions, yields small molecules (e.g., CO₂, H₂O) that are the final products of the oxidation process [11]. In certain cases, the etching mechanism is enhanced by UV photons (UV-induced etching), the photons acting synergistically with the reactive species, thereby accelerating the elimination rate of microorganisms (as in Fig. 3). This UV-induced chemistry, which occurs under nonequilibrium conditions, can result in the desorption of radicals and molecules, at both the intermediate and final stages of oxidation.

In both cases B and C, the by-products are removed through pumping and the gas phase is replenished with “new” reactive species.

We further assume that: (i) both reactive species and UV photons are present throughout the inactivation process; (ii) mechanisms A, B, and, when existing, C are active from the beginning to the end of the survival curve; (iii) all spores are ultimately inactivated by UV photon irradiation of their DNA material (mechanism A).

INACTIVATION PHASES AND THE THREE BASIC MECHANISMS OF PLASMA STERILIZATION

Spore inactivation at reduced pressure and under optimized conditions (shortest sterilization time) is the result of UV photons passing through the spore-protecting coats (Fig. 4) and destroying its DNA material. However, due to the average penetration depth of UV photons in matter, there are cases where not enough UV photons reach the genetic material to induce spore lethality within a reasonable time. This limitation depends on the number, thickness and chemical composition of the spore-protecting layers (Fig. 4) and on the location of its DNA material with respect to the surface exposed to UV radiation. The penetration of the UV photons is also restricted by eventual organic materials covering the spores (e.g., cell debris), and by the stacking (even partial) or aggregation of spores. The two scanning elec-

tron micrographs in Fig. 5 are examples of (untreated) spores that are stacked, clumped or wrapped within organic materials. In all these cases, erosion of the spore surface (mechanisms B and C above) makes it easier for the photons to reach the DNA material. Our starting point in interpreting plasma-sterilization survival curves was to note, from the literature, that spreading a given initial number of spores on a larger area yielded a higher mortality during the first phase [12]. The authors claimed this to be the result of a smaller number of spores per unit area exposed directly to the plasma. According to our observations, one should further consider that the stock suspension contains dead bacteria, proteins, and salts, which are distributed over a larger area with the more diluted suspension and are, therefore, less likely to cover living spores, and slow down the sterilization process. The results that we obtained with two dilutions of the same number of spores in the suspension confirm the above statements, as shown in Fig. 6: the higher the dilution, the shorter the D_1 and D_2 times. Figure 6 also shows that sterilization is reached in a shorter time with the more dilute suspension.

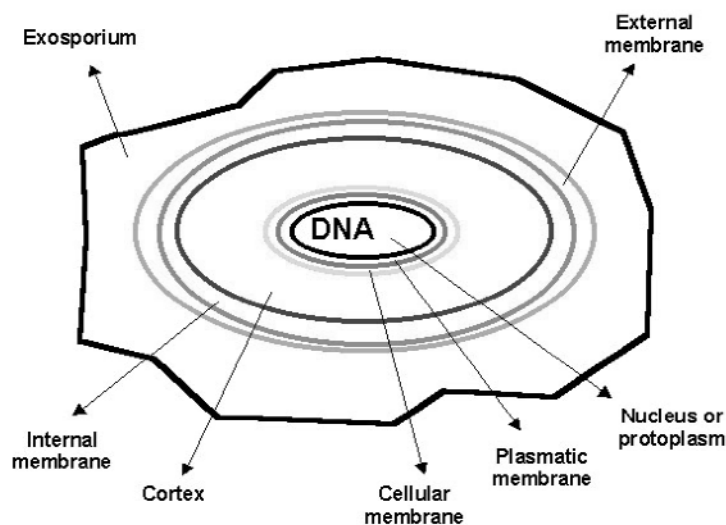


Fig. 4 Schematic representation of a bacterial spore with its genetic material (DNA) and protecting coats around it [8].

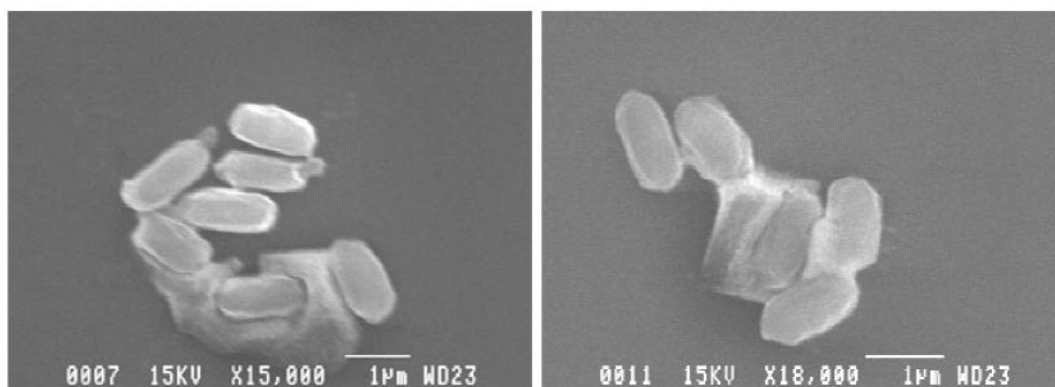


Fig. 5 Scanning electron micrographs showing stacked and clumped *B. subtilis* (*var. niger*) spores, some of which are embedded in organic material.

Given the fact that the characteristics of the first phase are qualitatively the same whether radicals from the gaseous phase are involved or not (Fig. 2) and given the limited penetration depth of UV photons, we suggest that the destruction by UV photons of isolated spores dominates during phase 1. Phase 2 would then be partly dependent on the erosion rate of the various materials (coats, debris, dead spores) covering still living spores. Phase 3 starts when the spores that were not inactivated during phases 1 and 2 have been sufficiently eroded or cleared from debris for the UV photons to finally kill them. This explains why the D_3 time is very often close to D_1 and why this third phase is observed immediately before sterilization is achieved. We believe that, in all cases, spore inactivation is ultimately the result of the DNA destruction by UV photons. This is supported by the work on UV germicidal lamps [13].

A summary of our interpretation of the survival curves obtained from plasma sterilization is schematized in Fig. 7. One can expect that survival curves with a single inactivation phase could be obtained provided all the spores were isolated, free from any covering material and their DNA material

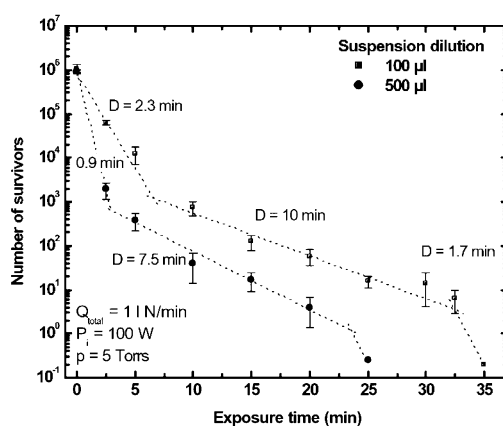


Fig. 6 Survival curve in the afterglow of an O_2-N_2 discharge at 0.7 % O_2 , showing the influence of diluting the spore suspension. The chosen percentage of O_2 maximizes the $NO\beta$ UV intensity under the present operating pressure and flow rate.

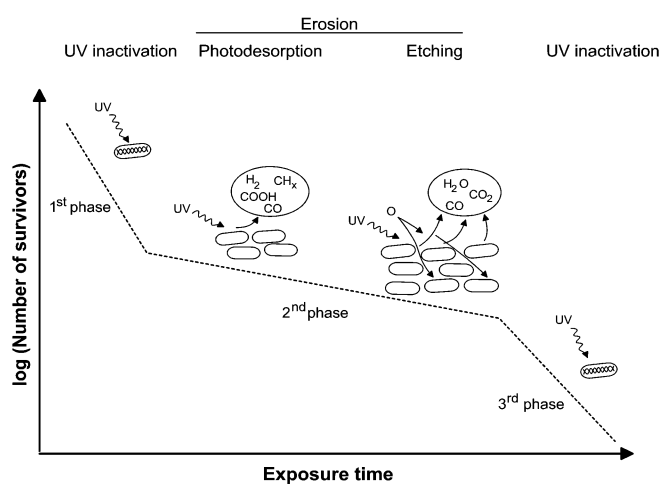


Fig. 7 Schematic illustration of the three-phase survival curve characterizing plasma sterilization, showing the mechanisms predominantly acting during each phase.

not too far from the spore exposed surface. Actually, experiments show (Fig. 6) that the number of isolated and uncovered spores, estimated from the intersect of phases 1 and 2, is close to 99.9 % of the initial population, leaving approximately 1000 “problematic” spores.

ADDITIONAL FACTS IN SUPPORT OF DNA DESTRUCTION BY UV IRRADIATION IN THE FIRST PHASE

Figure 8 shows the survival curves obtained by exposing to a UV excimer laser (248 nm) *B. subtilis* spores, deposited evenly onto planar aluminum-coated packaging surfaces (4-cm² boards) [14].

The cumulated UV dose (in joule) is actually proportional to exposure time. A biphasic curve is found, with an initial rapid inactivation phase followed by tailing. The authors hypothesize that the first phase of their survival curves is the result of the DNA destruction by direct UV irradiation of isolated spores, while the spores located within packaging crevices/pores are primarily responsible for the observed extensive tailing. Recall that the survival curve from the argon plasma afterglow shown in Fig. 2 (where it is assumed that only UV photons are acting) has a biphasic character too. A similar biphasic curve is also observed when using germicidal lamps where UV destruction of the DNA has been shown to be the basic mechanism of spore inactivation [13].

Obtaining a first phase dominated by the destruction of the DNA material by UV photons requires the discharge to be operated in the low or medium-pressure range (≤ 10 torr) where photon absorption by its emitting gas is not too high. In contrast, at atmospheric pressure, the initial slope of the observed biphasic curve (Fig. 9) is not the steepest one, indicating, as suggested by Kelly-Winterberg *et al.* [15], that the UV photons are not numerous enough to have a significant influence on direct inactivation of the microorganisms: photons emitted in the plasma at such a high pressure are very likely to be re-absorbed by their emitting gas, hence only a few of them reach the top spores. The nature of these two phases is still unclear.

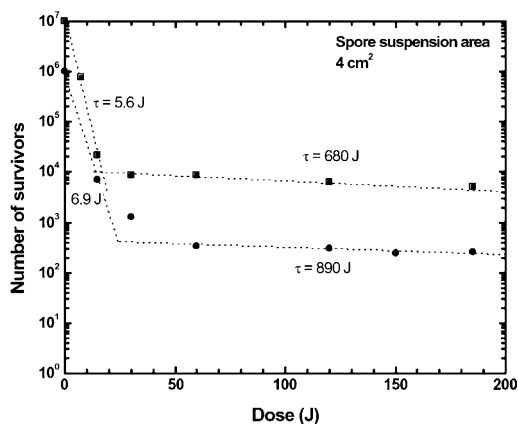


Fig. 8 Inactivation of *B. subtilis* spores of initial population 10^7 (■) and 10^6 (●), deposited onto planar aluminum coated packaging surfaces, by UV irradiation from an excimer laser (248 nm). τ is the dose required to inactivate a decade of spores [14].

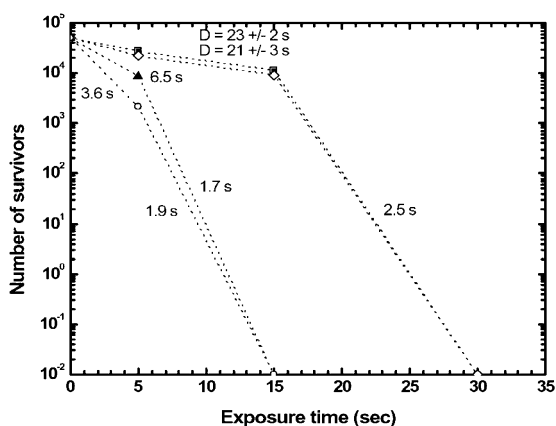


Fig. 9 Survival curve of *S. aureus* and *E. coli* cells (5.0×10^4) seeded on polypropylene samples and exposed to a DBD-type discharge in air at atmospheric pressure. *S. aureus* cells exposed unwrapped (\diamond) and in semi-permeable bags (\blacksquare), *E. coli* cells exposed unwrapped (\circ) and in semi-permeable bags (\blacktriangle) [15].

COMMENTS ON THE SECOND AND THIRD PHASES

Because the second phase, which has the slowest kinetics, lasts longer than phase 1 (at least a factor of 3 longer), the level of erosion attained during this phase is more important. This second phase ends when the inactivated spores and debris of all kinds standing on top of living spores have been sufficiently eroded to allow an easier access of the UV photons to the spore DNA, leading to the faster inactivation kinetics characterizing phase 3. The tailing of the second phase relatively to the first phase in the case of plasma inactivation (for instance, Fig. 2) is not as extensive as with the UV laser (Fig. 8) because there is no shadowing effect related to the plasma photon source: excited atoms or molecules are carried as a gas into the crevice or pore where eventually UV photons will be emitted. The probability that a photon be emitted within a crevice in the plasma case can be low (e.g., because of deexcitation of the molecule as it hits the edge of the crevice or pore), but not as close to zero as finding there a photon coming from a UV lamp or laser

The third step starts only after some delay with respect to the beginning of the second phase. This delay is the shortest when reactive species such as oxygen atoms are present and when the UV emission intensity has been maximized [4,8]. In the presence of oxygen atoms, but with a less intense UV photon flux, phase 3 starts after a longer time. In the absence of reactive species (e.g., O), only intrinsic photodesorption (mechanism B) can take place, hence a still much longer delay time before achieving sterilization (case of pure argon in Fig. 2).

Intrinsic photodesorption (mechanism B) is the only possible erosion mechanism with Hg lamps or UV lasers in ambient air, as well as with the discharge in pure argon (Fig. 2). The D_2 time in Fig. 2 becomes shorter when oxygen is added to the argon gas, in accordance with our assumptions: erosion is faster when etching comes into play in addition to photodesorption. Furthermore, the D_2 time decreases when the substrate temperature is increased [12] which most probably implies that mechanism C is then thermally activated.

Reduction in the microorganism size inherent to plasma sterilization has been reported earlier, supporting the existence of an erosion process. Laroussi *et al.*, {atmospheric-pressure, contact with a dielectric barrier discharge (DBD)-type plasma sustained in a mixture of helium and air [16]} show micrographs where the destruction of *E. coli* is almost complete, while Lerouge *et al.*, (reduced-pressure, contact with a pulsed microwave-sustained plasma in O_2 [17]) obtain an important ($\approx 50\%$) reduc-

tion of the spore area of *B. subtilis*. In our flowing afterglow system (conditions as in Fig. 6, lower dilution case), the spore area is reduced by approximately 15–20 % only. We attribute this lower degree of erosion to a lower concentration of reactive species in the gaseous phase.

STERILIZATION BY DIRECT CONTACT WITH PLASMA OR IN THE FLOWING AFTERGLOW: ADVANTAGES AND SHORTCOMINGS

Plasma sterilization can be achieved either in the discharge itself or in its afterglow. Compared to the discharge itself, the afterglow contains relatively few charged particles, being essentially comprised of neutral atoms, radicals and molecules, some of which are in an excited state.

The main advantages of using the afterglow for sterilization purposes instead of the discharge itself can be summarized in the following points [2]: (1) with high-density plasmas such as those produced by microwaves (unless operated in a pulsed regime), the gas temperature in the discharge itself can reach a few hundreds °C, while it can be made less than 50 °C in the corresponding afterglow, an important characteristic when dealing with heat-sensitive materials; (2) under direct plasma exposure, the treated surfaces have some chances of being altered by the impact of the (positive) ions accelerated in the sheath. There is no sheath in an afterglow; (3) there is no essential need to operate in the discharge itself since, as shown by recent studies, it is the neutral species, not the charged ones, that play the major role in plasma sterilization [18]; (4) the presence in the processing region of the E-field sustaining the plasma can induce local heating in nondielectric devices, hence possible damage to them; 5) the afterglow can fill larger chamber volumes at lower costs than the corresponding glow discharge. However, sterilization time is usually much shorter in the discharge itself than in its afterglow. In both operating modes, as a rule, the hydrodynamics of the gas flow is critical in order to ensure that enough photons and reactive species reach all parts of the devices to be sterilized, everywhere in the chamber.

CONCLUSIONS

Plasma inactivation is characterized by the existence of two or three distinct phases in the survival curves of a given type of spores. These phases correspond to changes in the dominating kinetics of spore inactivation as a function of exposure time. In the case of medium and low-pressure discharges, the basic process is the DNA destruction of the spore by UV irradiation, preceded in some cases by erosion of the microorganism through intrinsic photodesorption and etching (eventually enhanced by UV radiation). These elementary mechanisms clearly set plasma sterilization apart from all other sterilization methods. In that respect, the inactivation of pathogenic prions, which have no genetic material, could certainly be achieved through full erosion of these proteins [19]; however, it could be that intense UV radiation, acting synergistically with oxygen atoms, causes substantial damage to these proteins, thereby avoiding the need for a full erosion. More recently, and not included in the above presentation, we have shown that a better understanding of the inactivation process is attained by using the concept of fluence (number of photons actually interacting with the genetic material over a given period of time) that depends on the absorption coefficient and penetration depth of photons in the spore material [20]. Fluence is actually more appropriate than the incident flux of UV photons since DNA inactivation results from a given (statistical) number (or dose) of damaging hits on its strands [20].

Efficient plasma sterilization at reduced pressure in O₂ containing mixtures under flowing afterglow conditions depends essentially on maximization of the UV emission intensity. Similar results are expected when the microorganisms are directly exposed to the corresponding discharge. Etching in presence of oxygen atoms provides a faster erosion of the protecting layers of microorganisms than simply photodesorption, only the latter being possible with Hg lamps. Furthermore, sterilization using UV lamps and lasers suffers from shadowing effects (which includes the difficulty to sterilize in crevices), in contrast to gas plasma sterilization where the UV photon is brought to the appropriate site by its emitting atom or molecule.

An important shortcoming of plasma sterilization is its dependence on the actual “thickness” of the microorganisms to be inactivated since the UV photons need to reach the DNA. Any material covering the microorganisms, including packaging, will slow down the process.

Further research must establish the efficacy of plasma sterilization of small-diameter, long cylinders, such as endoscopes and examine to what extent devices can be sterilized in their packaging. It also remains to determine the degree of damage to surfaces of different materials subjected to the discharge glow or afterglow.

REFERENCES

1. M. Moisan, J. Barbeau, J. Pelletier. *Le vide: Sci. Tech. Appl.* **299**, 15–28 (2001).
2. M. Moisan, J. Barbeau, S. Moreau, J. Pelletier, M. Tabrizian, L'H. Yahia. *Int. J. Pharm.* **226**, 1–21 (2001).
3. M. Moisan, J. Barbeau, J. Pelletier, N. Philip, B. Saoudi. 13th Int. Coll. Plasma Processes (SFV), Antibes (2001); *Le vide: Sci. Tech. Appl.* Numéro spécial: Actes de Colloque, pp. 12–18 (Mai 2001).
4. N. Philip, B. Saoudi, J. Barbeau, M. Moisan, J. Pelletier. 13th Int. Coll. Plasma Processes (SFV), Antibes (2001); *Le vide: Sci. Tech. Appl.* Numéro spécial: Actes de Colloque, pp. 245–247 (Mai 2001).
5. M. Laroussi. *IEEE Trans. Plasma Sci.* **24**, 1188–1191 (1996).
6. H. W. Hermann, I. Henins, J. Park, G. S. Selwyn. *Physics Plasmas* **6**, 2284–2289 (1999).
7. S. Cariou-Travers and J. C. Darbord. *Le vide: Sci. Tech. Appl.* **299**, 34–46 (2001).
8. S. Moreau, M. Moisan, M. Tabrizian, J. Barbeau, J. Pelletier, A. Ricard, L'H. Yahia. *J. Appl. Phys.* **88**, 1166–1174 (2000).
9. S. Lerouge, A. C. Fozza, M. R. Wertheimer, R. Marchand, L'H. Yahia. *Plasmas Polymers* **5**, 31–46 (2000).
10. A. Ricard, M. Moisan, S. Moreau. *J. Phys. D: Appl. Phys.* **34**, 1203–1212 (2001).
11. J. Pelletier. *Agressologie* **33**, 105–110 (1993).
12. S. Hury, D. R. Vidal, J. Pelletier, T. Lagarde. *Lett. Appl. Microbiol.* **26**, 417–421 (1998).
13. I. O. Soloshenko, V. A. Khomich, V. V. Tsiolko, I. L. Mikhno, A. I. Shchedrin, A. V. Ryabtsev, V. Yu. Bazhenov. *Proc. 14th Int. Symposium Plasma Chemistry*, Prague, 2551–2556 (1999).
14. K. Warriner, G. Rysstad, A. Murden, P. Rumsby, D. Thomas, W. M. Waites. *J. Appl. Microbiol.* **88**, 678–685 (2000).
15. K. Kelly-Wintenberg, T. C. Montie, C. Brickman, J. R. Roth, A. K. Carr, K. Sorge, L. Wadsworth, P. P. Y. Tsai. *J. Ind. Microbiol. Biotechnol.* **20**, 69–74 (1998).
16. M. Laroussi, I. Alexeff, W. L. Kang. *IEEE Trans. Plasma Sci.* **28**, 184–188 (1999).
17. S. Lerouge, M. R. Wertheimer, R. Marchand, M. Tabrizian, L'H. Yahia. *J. Biomed. Mater. Res.* **51**, 128–135 (2000).
18. V. A. Khomich, I. A. Soloshenko, V. V. Tsiolko, I. L. Mikhno. *Proc. 12th International Conference on Gas Discharge and their Applications*, Greifswald, **2**, 740–744 (1997).
19. M. Moisan, S. Moreau, M. Tabrizian, J. Pelletier, J. Barbeau, L'H. Yahia. “Système et procédé de stérilisation par plasma gazeux à basse température”, PCT/CA00/00623 (patent application) (2000).
20. N. Philip, B. Saoudi, M.-C. Crevier, M. Moisan, J. Barbeau, J. Pelletier. *IEEE Trans. Plasma Sci.* **30**, (4) (2002). In press.