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Review

Low-temperature sterilization using gas plasmas: a review of the experiments and an analysis of the inactivation mechanisms

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Abstract

Utilizing an ionized gas (plasma) to achieve sterilization is an alternative to conventional sterilization means as far as sterilization of heat-sensitive materials and innocuity of sterilizing agents are concerned. The literature on plasma sterilization is reviewed. A major issue of plasma sterilization is the respective roles of UV photons and reactive species such as atomic and radicals. Insight into this matter is obtained by analyzing the survival curves of microorganisms. In contrast to classical sterilization where such plots show a unique straight line, plasma sterilization yields survival diagrams with two or three different linear segments. Three basic mechanisms are involved in the plasma inactivation of microorganisms: (A) direct destruction by *UV irradiation* of the genetic material of microorganisms; (B) erosion of the microorganisms atom by atom, through *intrinsic photodesorption* by UV irradiation to form volatile compounds combining atoms intrinsic to the microorganisms; (C) erosion of the microorganisms, atom by atom, through *etching* to form volatile compounds as a result of slow combustion using oxygen atoms or radicals emanating from the plasma. In some cases, etching is further activated by UV photons, increasing the elimination rate of microorganisms. These mechanisms make plasma sterilization totally different from classical sterilization techniques and suggest its use to inactivate nonconventional infectious agents such as the abnormal prions.

Keywords: Sterilization; Gas plasma; Survival curve; Etching; Photodesorption; UV irradiation; Spore

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1. Introduction

The advent of polymeric medical devices has stimulated users and developers to look for new methods to sterilize heat-sensitive materials. The most common technique used nowadays for such a purpose relies on ethylene oxide (EtO). The first version of this method had to be modified because of environmental regulations on chlorofluorocarbons (CFCs) that were added to EtO to reduce its flammable properties (Ernest, 1995). Although 100% EtO sterilizers, which no longer use CFCs, have been developed, there are still many questions concerning the carcinogenic properties of the EtO residues adsorbed on the materials after processing (Steelman, 1992a; Holyoak et al., 1996; Zhang et al., 1996). There are also worries about the safety of operators when opening the sterilizer before the end of the vent time, which is much longer than the actual sterilization time (Steelman, 1992b). Although the most common low-temperature technique currently used calls for gaseous EtO, liquid formaldehyde and glutaraldehyde, which have noxious properties too, are also employed in many medical sterilization installations (Ehrenberg et al., 1974). Another interesting technology is the gamma irradiation process, but it is costly and its safe operation requires an isolated site; moreover, besides damaging to some extent the surface of the objects like any sterilization method, gamma irradiation also affects the bulk properties of the polymers being treated, as it breaks bonds and cross-linked chains within the volume of the material (Henn et al., 1996).

The various limitations of the above sterilization methods have fostered the development of alternative techniques. The ideal sterilizer should provide:

1. a sterilization time shorter or at least not longer than the actual processing time required with steam autoclave and dry heat methods (approx. 60 min);
2. an equal or lower processing temperature than that encountered with EtO (55 °C or less);
3. the possibility of dealing with a wide range of materials and objects;
4. harmless operation for operators, patients and materials.

The use of a gas plasma offers an original alternative to sterilization because of the intrinsic properties of this medium. The method consists in exposing microorganisms to species stemming from an electrical discharge in a gas. In the pure form of this method, the gas or gases involved have no biocidal effect unless they are activated by the electrical discharge; these reactive species are no longer present a few milliseconds after the electric field has been turned off, which means that there is no need for vent time and, therefore, very little danger for the personnel. The operating conditions of the plasma have to be set for an efficient inactivation of the microorganisms, while minimizing the damage to the materials subjected to the treatment (Anderson, 1989; Lerouge et al., 2000a).

The present article reviews and analyzes the work achieved on gas plasma sterilization. Until recently, very little was known about the inactivation mechanisms of microorganisms with such a technique. The specific characteristics of the *survival curves*, ie the logarithm of the number of survivors as a function of exposure time to the plasma, show, as we will see, that the kinetics of such a process is quite different from that of classical sterilization methods. Actually, the analysis of the survival curves together with the identification of the species active in the killing of spores led us to the inactivation mechanisms and provided indications as to the advantages and limitations of this method (Moisan et al., 2001a,b).

The presentation is organized as follows. In the coming section (Section 2), we recall facts and data about gas plasmas. Then Section 3 starts with a short historical summary of the first steps in plasma sterilization and, afterward, concentrates on the identification of the species involved in the inactivation process of microorganisms. Section 4 deals with the detailed analysis of the survival curves, providing experimental evidence in support of the explanation proposed. Section 5 is the conclusion.

2. Definition and characteristics of a gas plasma

2.1. Gas plasmas, ionized gases and electrical discharges

Gas plasmas, simply termed plasmas by physicists who don't fear ambiguity with the blood component, can be considered as the fourth state of matter, following by order of increasing energy, the solid state, the liquid state and the gaseous state. This fourth state, as encountered in stars, is strictly speaking a gas made of ions and free electrons only. However, the plasmas employed for sterilization are much colder and are actually *ionized gases*: besides ions and electrons, ionized gases also consist of uncharged particles, such as atoms, molecules and radicals (atoms or assembly of atoms with unpaired electrons, therefore chemically reactive, eg O and OH, respectively), collectively called *neutrals*. In our presentation, as is common practice, we shall use the term plasma to designate as well an ionized gas, ignoring the above distinction.

Ions and neutral atoms (molecules) can be in an excited state, ie they can have *internal energy*, this energy being zero when these particles are in their ground state. Excited particles can de-excite (lose their internal energy), either spontaneously by emitting a photon or through collisions with other particles or a surface. Collision with a surface can lead to the physical sputtering of their elements or to chemical reactions, such as oxidation, resulting in some cases in a volatile compound (eventually pumped out) formed between the incoming (adsorbed) atom (radical, molecule) and a surface atom. Photons emitted by excited species can also induce chemical reactions on a surface: UV photons are particularly efficient in this respect (Lerouge et al., 2000b).

Man-made plasmas are usually produced by subjecting a gas (or gases) to an electric field (say, between two electrodes), either of constant (direct-current, d.c., field) or alternating amplitude (usually high frequency field), hence their designation as *electrical discharges*. The electric field E accelerates the charged particles, essentially the electrons since the ions are much heavier, and the E-field energy is ultimately communicated to the plasma through collisions of the electrons with the heavy particles (Delcroix and Bers, 1994; Moisan and Pelletier, 1999).

2.2. The glow and the flowing afterglow of a plasma

As a rule in electrical discharges, most of the gas volume is quite luminous, which is why one speaks of a *glow discharge*. When the discharge takes place in a flowing gas, some of the species produced in the glow region can be carried away into an electric field-free vessel, and one obtains what is called a *flowing afterglow*.

Plasma sterilization can be achieved either in the glow or in the afterglow region. Compared to the glow region, 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 species of interest are then short-lived particles, for instance atoms like O and N, and excited molecules such as NO in an O_2–N_2 gas discharge. Short-lived particles, therefore, need to be carried to their point of use rapidly enough, hence a minimum value for the gas flow rate.

The main advantages of using the afterglow for sterilization purposes instead of the discharge itself (Moreau et al., 2000) can be summarized in the following points: (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 Celsius, 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*. The sheath is the interfacial region between the plasma and any surface immersed in it where the ion density exceeds that of electrons (both densities are equal in the plasma). This non-neutrality results from a potential difference between the plasma and the surface, which accelerates ions toward the surface. 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 that play the major role in plasma sterilization (Khomich et al., 1997). (4) The presence in the processing region of the E-field sustaining the plasma can induce local heating in non-dielectric 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, one must ensure that enough active species reach all parts of the devices to be sterilized, everywhere in the chamber.

2.3. Cold plasmas

The plasmas most commonly used for sterilization purposes are known as *cold plasmas* with reference to their gas temperature. This is because heavy particles (neutrals and ions) in these plasmas have temperatures much lower (typically at least ten times) than the temperature (average energy) of the electrons. At pressures typically below 10 Torrs (≈ 10 mbar), cold plasmas are characterized by a mean electron energy in the range 1–5 eV ($1\ \text{eV} = 1.6 \times 10^{-19}$ J or 11 600 K). Therefore, it is the electrons that break molecules, and excite or ionize atoms and molecules of the discharge gas without requiring the heavy particles to be as energetic (Boucher (Gut), 1985), hence a lower energy cost and the possibility of using glass and metal discharge vessels without a heavy cooling. With some kinds of pulsed discharges (eg corona and dielectric barrier discharges, DBDs: for details, see Eliasson and Kogelschatz, 1991), the gas temperature can even be as low as room temperature. As a rule, the temperature of the discharge gas increases with pressure (because of the increasing number of elastic collisions of electrons with heavy particles) and, depending on the gas, can exceed a few thousands degrees at atmospheric pressure unless a pulsed discharge is used.

Low-temperature sterilization *under direct plasma exposure* can be realized by running d.c. discharges (which means constant E-field intensity as a function of time), radio-frequency (RF; typically 1–100 MHz) and microwave ($\geqslant 300$ MHz) discharges under low current and low power conditions, respectively. It can also be achieved by sustaining microwave discharges in a pulsed mode (Griffiths and Raybone, 1992; Lerouge et al., 2000c) or by using very low frequency E-field (eg 1–10 kHz), as required to operate corona discharges and DBDs (Sbai, 1991; Kelly-Wintenberg et al., 1998; Kuzmichev et al., 2000; Laroussi et al., 2000).

3. Operating conditions and active species of electrical discharges

We start with a brief historical summary of the early experiments on microorganism inactivation by plasma.

3.1. The first steps in plasma sterilization

The first report on plasma as a sterilizing agent was by Menashi in a 1968 patent (Menashi, 1968). The apparatus used a pulsed RF field to achieve an argon plasma at atmospheric pressure and sterilize the inner surface of vials. For this purpose, the RF field was imposed in the bottle by a straight wire and a coil wrapped around the outside of the vial acted as the reference electrode to close the RF circuit (corona-type discharge). Menashi was able to sterilize vials containing 10^6 spores in times of less than one second. It was found necessary that the plasma contacted the interior surface of the vial and, for this reason, it was thought that the biocidal action was due to intense heating of the spores in a time too short to appreciably heat the glass container (this mechanism was later termed microincineration by Peeples and Anderson, 1985a).

Further patents by Ashman and Menashi (1972) as well as by Boucher (Gut) (1980) and Bithell (1982) showed that an electrical discharge in an appropriate gas (or gases) could lead to sterilization. Fig. 1 shows an example of the sterilizer prototypes described in these patents. The samples are inserted within a chamber, afterward evacuated with a fore pump to some 10–40 mTorrs (1–5 Pa) and subsequently filled with gas set at the required pressure, typically 0.05–2 Torr

(5–300 Pa), using pressure gauges. Gas discharge is then achieved by applying an RF field (generally 13.56 MHz, a worldwide authorized frequency for industrial, scientific and medical, ISM, applications) to the gas by means of a coil (or parallel plates) located on the outside of the (dielectric) chamber. A wattmeter is employed to indicate the power transmitted to the gas while an impedance matching network enables the operator to minimize the power reflected, toward the power generator, from the RF field applicator. Boucher (Gut) (1980) also realized sterilization with a microwave-sustained discharge at 2450 MHz (the microwave oven frequency), another ISM authorized frequency. The first systems reported in the scientific literature were of the same nature as those of the patents. More elaborated plasma arrangements were later developed. For example, to sterilize the inner part of vials, a microwave field was applied from the outside (in contrast to Menashi's system where the inside electrode could generate particulates) and the discharge was initiated by a laser pulse; sterilization times of a few seconds were reported for a 200 W plasma (Tensmeyer et al., 1981).

Most of these early experiments used inert gases, such as argon or helium. However, Ashman and Menashi (1972) added halogens such as chlorine, bromine and iodine within the sterilization chamber to increase the efficacy of the process. Boucher (Gut) (1980) seeded his carrier gas with aldehydes. Instead of rare gases as the carrier gas, Jacobs and Lin (1987) directly used hydrogen peroxide, a sterilant agent, in an aqueous solution in a two-step process: (1) injection and contact of H_2O_2 with the objects to be sterilized; (2) application of an RF discharge ensures that no toxic residues remain on the sterilized items.

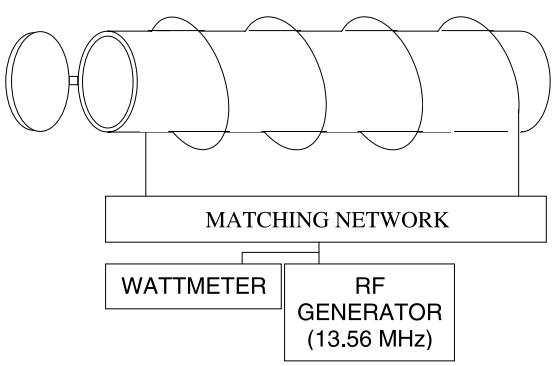

Fig. 1. Schematic of an early typical apparatus for plasma sterilization (after Boucher (Gut) 1980).

3.2. Discharge operating conditions and identification of active species

Discharge operating conditions include the nature, composition, pressure and flow rate of the gas(es) used, the discharge vessel configuration and dimensions, and the applied field frequency. The power absorbed in the plasma is to be considered as a separate parameter (Zakrzewski and Moisan, 1995); also note that the absorbed power per unit volume (power density), not the absolute value of absorbed power, is meaningful when comparing different experimental arrangements. In this section, we list the first observations relating the active species with the operating conditions.

Plasma sterilization experiments have been conducted in essentially three pressure domains, which we designate as low pressure (1–10 mTorr range), medium pressure ($\approx$0.1–10 Torr) and atmospheric pressure. In the medium pressure range, there have been experiments under both direct plasma and afterglow exposures. Most initial experiments (Section 3.1) and those reported in the present section have been achieved in the medium pressure domain.

3.2.1. Role of the gas or the gas mixture

Boucher (Gut) (1985) observed that some gases (e.g. CO_2) were more efficient than others (eg argon) to inactivate bacterial spores. He also claimed that spores presoaked for one hour in water are easier to kill. Ratner et al. (1990) showed that plasma sterilization is efficient with most discharge gases (O_2, N_2, air, H_2, halogens, N_2O, H_2O, H_2O_2, CO_2, SO_2, SF_6, aldehydes, organic acids,…), whatever the type of discharge.

3.2.2. Power density in the discharge

In his 1980 patent, Boucher mentioned that the RF power density should be at least 1 mW/cm^3. Boucher (Gut) (1985) further reported that the

sterilization efficacy increased with the (RF) power density absorbed in the discharge.

3.2.3. The role of UV

The possible action of UV irradiation on microorganisms was already raised quite well by Boucher (Gut) (1980) in his extensive patent description: "The UV high energy photons (3.3 to 6.2 eV) will produce strong cidal effects because they correspond to a maximum of absorption by DNA (deoxyribonucleic acid) and other nucleic acids. However, in the case of spores that can reach one millimeter in diameter, photon energy could be quickly dissipated through the various spore layers and this may restrict photochemical reactions to outer coats… The depth of action of UV photons is restricted to a one micrometer layer. The maximum observed depth for photochemical action in non-oxygen plasmas was 10 μm in the case of polyethylene gelation or ablation. In other words, the photon energy is rather restricted to thin layer surface modifications (changes in plastics wettability and bondability) and will, therefore, be more efficacious when dealing with the smallest non-sporulated bacteria. In the case of high resistance spores, the photonic action may contribute to partial alteration of the disulphide rich proteins coat and thus facilitate the diffusion of free radicals, atoms, or excited molecules inside the core region" (Boucher (Gut) 1980). Boucher's point concerning the limited penetration depth of UV is right; it is of the order of the spore dimensions which, as a rule, do not exceed 1–3 μm, but spores can be stacked or aggregated. At any rate, quite surprisingly, Boucher (Gut) (1985) later on concluded that "the sporicidal action of the plasma is not greatly affected by the presence or absence of UV radiation".

Tensmeyer et al. (1981), already cited, suggested that the sporicidal action of their plasma was probably due to the radiation it emits since the discharge did not appear to contact the vial walls, but they did not investigate the type and level of photon emission. Peeples and Anderson (1985a) expanded on the work of Tensmeyer et al. (1981) by improving the experimental arrangement of their laser-initiated microwave-sustained discharge (longer plasma duration and reduced power requirement). In a companion paper, Peeples and Anderson (1985b) showed that the laser 'spark' by itself was insufficient for any sporicidal effect, most likely due to its extremely short duration (< 5 μs), and therefore inferred that the microwave-sustained discharge was responsible for such an effect. Monitoring the UV emission intensity from their atmospheric-pressure air plasma in the 220–290 nm range, they found that it was about twice the output of a germicidal Hg lamp; from such data, they calculated that their plasma should be able to kill 47 decades of *Bacillus subtilis* within only 0.2 s! Nonetheless, on an experimental basis, their system could not provide sufficient depyrogenation for serum vials since the level of endotoxin (*Escherichia coli*) destroyed by the plasma was not significant. In fact, photographs taken by them indicated that contact of the vial wall by the plasma was lost one second after plasma ignition. They finally concluded that, even though there was a copious amount of UV radiation emitted by the plasma, the depyrogenation action of the plasma was not the result of the UV light generated by the microwave sustained discharge, but of microincineration.

Up to this point in our survey of the literature (1985), the actual role of UV radiation in plasma sterilization was far from being acknowledged or understood.

3.2.4. The role of oxygen atoms

Nelson and Berger (1989) showed on *B. subtilis* and *Clostridium sporogenes* that an O_2 plasma could be very efficient as a biocidal medium. Plasma was provided by a reactive-ion etching (RIE) system, where the discharge is achieved between two parallel conducting plates with an RF (13.56 MHz) field. The gas pressure is not specified, most likely in the 0.02–0.2 Torr range. This system accelerates the ions as they drift toward the electrodes on which, presumably, the spores were located. With 200 W, the population of *B. subtilis* was reduced by more than 3.5 $\log_{10}$ in 5 min. *B. subtilis* inactivation with an O_2 plasma had been reported earlier by Fraser et al. (1976), but with a lower efficacy since it required 300 W and 15 min exposure, most probably because of different operating conditions.

3.2.5. Influence of the type of microorganisms

To assess the sterilizing efficacy of plasmas, one should test the most resistant microorganisms. Initially, as mentioned by Boucher (Gut) (1985), to legally support a sterilization claim in the gas phase, the American Environmental Protection Agency (EPA) required tests to be made with two types of aerobic and anaerobic spores (*B. subtilis* and *Clostridium sporogenes*, respectively), while the current standard procedures for checking autoclaves and EtO sterilizers were based on the inactivation of *B. subtilis* and *B. stearothermophilus*. However, Boucher showed that *B. stearothermophilus* was, in fact, a bit more resistant than *B. subtilis* to plasma sterilization (contact with an air plasma at pressures ≈ 0.6–1.1 Torr), as was later on also reported by Kelly-Wintenberg et al. (1998, atmospheric-pressure air plasma). In contrast, Hury et al. (1998, low-pressure O_2 plasma) found that *B. stearothermophilus* was easier to inactivate than *B. subtilis*. As for Krebs et al. (1998), they observed that with hydrogen-peroxide plasma-based systems (Section 3.4), *B. stearothermophilus* was more resistant than *B. subtilis*. Finally, comparing *B. stearothermophilus* (a spore-forming Gram-positive aerobic rod) and *E. coli* (a Gram-negative facultative rod), Baier et al. (1992), using a medium-pressure plasma (35 MHz discharge in argon within a glass tube), observed that Gram-negative bacteria are more resistant to the process, due to their extra proteins and their lipopolysaccharide walls. Clearly, to be on the safe side, a plasma sterilizer should be initially tested with *B. subtilis*, *B. stearothermophilus* and a Gram-negative bacterium such as *E. coli*.

3.2.6. Sterilization of packaged objects

Bithell (1982) showed that sealed packages could be placed in a plasma (O_2 RF-discharge at 40 Pa 0.3 Torr) and the article contained therein sterilized through the package. Sterilizing packaged items raises the question of the active species that can go across the pores of a packaging material: a priori, excited atoms (molecules) and UV photons should not go through without their concentration and flux, respectively, be somehow reduced.

3.3. First attempts at modeling the inactivation process

Following the above first experiments, a series of interesting hypotheses on the sterilization mechanisms of microorganisms by a gas plasma began to emerge. Nelson and Berger (1989) summarized the situation as follows: "The specific physiochemical mechanisms responsible for inactivation are not completely understood. Physical sputtering of the outer walls of microbes, chemical degradation by active species in the plasma, and UV light given off by the plasma have all been attributed to the microbiocidal effects of [plasma sterilization]".

The first quantitative description of the mechanism of plasma sterilization came from Pelletier (1993). It is based on erosion, atom by atom, of the microorganism material: gaseous species from the plasma get adsorbed on the microorganism in such a way that volatile compounds are formed, which are pumped out. Such an erosion mechanism of surfaces is known as *etching* in the plasma processing of chips in microelectronics.

His model assumes, to a first approximation, that the microorganisms are macromolecules mainly made up of carbon, hydrogen, oxygen and nitrogen. Among these elements, carbon is the only one not self-associating to make a volatile molecule, and it must therefore form volatile compounds with other atoms in order to be removed from the surface. In that respect, assuming the chemistry on the surface to occur at thermodynamic equilibrium and considering gas temperatures below 500 K, he showed that in oxygen-based plasmas, from atmospheric pressure down to pressures as low as 10^{-3} Torr (0.13 Pa), the only (stable) volatile end-product from the combustion of carbon is CO_2, implying that the amount of CO released should be negligible. Then, considering the sequential adsorption and desorption mechanisms of O_2 and O on a surface, he showed that only oxygen atoms can adsorb in the appropriate nearest-neighbor positions in order to efficiently form CO_2 molecules with carbon atoms of the macromolecule. Therefore, this slow combustion of carbon and, as a result, the efficacy of the sterilization process, should increase with

the increase of oxygen atoms in the gas phase, until they saturate the surface. Pelletier further showed theoretically that the formation of CO_2 and its subsequent desorption as a volatile compound increases with the temperature of the surface, following an Arrhenius (activation) law: the higher the temperature of the object, the faster should then be the killing of bacteria.

Pelletier (1993) did not consider other discharge gases, but conjectured that, in general, the chemically active species from the gaseous phase are radicals (because of their unpaired electrons(s)), excited molecules, and ions. No mention of the role of UV was then made, although it was known that UV photons can take part in the erosion of polymers (for example, Pons et al., 1994; Wertheimer et al., 1999), yielding CO and CO_2 as possible by-products through *photodesorption*, a non-equilibrium chemistry mechanism. The action of ions was discounted on the basis that they would not go across the wrapping paper used to store the sterilized objects.

3.4. Further experimental results

Hury et al. (1998) reported the inactivation of spores on objects immersed in argon and oxygen-based (O_2, CO_2 and H_2O_2) microwave-sustained plasmas, in the low-pressure (mTorr) range. They used *B. cereus*, *B. subtilis*, *B. pumilus* and *B. stearothermophilus* as test spores. They found that: (1) *B. stearothermophilus* was more easily inactivated than the three other types of spores, which showed similar survival curves; (2) H_2O_2 and CO_2 discharges have the highest inactivation rate and the argon discharge has the lowest. They claimed that degradation of the microorganisms was likely obtained by their slow combustion with the oxygen atoms or oxygen-containing radicals present in the plasma. At or near room temperature, this combustion is assumed to produce CO_2 and H_2O.

Their survival curve, shown in Fig. 2, is particularly noteworthy. The linear least-square fits that we made on their data suggest the existence of three distinct slopes. To characterize these slopes, we use the time D required to decrease a given population of spores by a factor of 10 (90% reduction). The smallest D value is that of the first slope and the largest one is that of the second slope. The two sets of curves are for the same total number of spores, but for a surface of exposure in a ratio of three as a result of dilution of the suspension. The difference between these two survival curves is essentially related to the extent of the first inactivation phase: according to the authors, more spores are killed in the first phase when the number of stacked spores is reduced by turning to a lower surface density of spores.

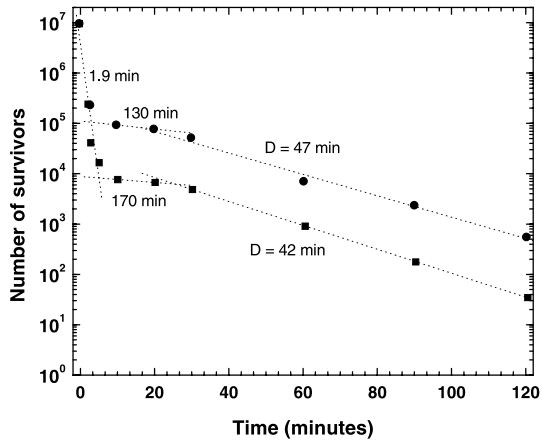

Fig. 2. Survival curve of *B. subtilis* spores exposed to a 4 mTorr (5 Pa) CO_2 plasma showing the influence of the spore surface density for the same total number of spores: (●) high surface density, (■) low surface density (after Hury et al., 1998; the linear least-square fits were made by us).

Kelly-Wintenberg et al. (1998) used an atmospheric-pressure air discharge to inactivate various microorganisms, including bacteria and endospores. Plasma was achieved between parallel plates with an applied low-frequency field in the 1–8 kHz range (DBD-type discharge), yielding a relatively low ion-electron density ($\approx 10^{10}$ cm^{-3}) (given the fact that this is an atmospheric-pressure discharge) and, accordingly, a gas discharge temperature close to the ambient. The samples were laid flat and taped down on the surface of the insulator covering the bottom electrode, and were therefore in contact with the plasma, directly or through the pores of their packaging material. Air flow was provided to promote plasma uniformity. The inactivation time is short; typically, they ob-

tain a reduction by more than 5 $\log_{10}$ of *B. subtilis* in 5 min with an initial population of 10^6 spores. The possible active species are listed as: radicals such as O_2^-, hydroxyl, OH^-, NO^-, hydrogen peroxide, and ozone. They discounted any significant action of the UV photons by arguing that the bag would have blocked much of them (presumably, a discharge was not achieved in the bag).

Their survival diagram is of particular interest. As shown in Fig. 3, they obtain a *biphasic* curve. The first step is claimed to be exponential, while "it was not clear that the second curve was exponential". In contrast to Hury et al. (1998), the first phase has the largest *D* time. The data points in their figure are not numerous enough for us to proceed to a more detailed analysis of their results. Using pure helium as the discharge gas instead of air, they observed that the inactivation process was much less efficient (no time duration is however stated for full inactivation in this case). Concerning the reasons for a biphasic curve, they "hypothesize that during the first killing phase, toxic active species are concentrating and generating alterations at the membrane level. Hence, the differences in D_1 values of different organisms reflect the differences in surface and membrane structure coinciding with varied resistance to these active species. Once the concentration of active species is sufficient for lethality, the second phase is very rapid, resulting in irreversible damage and lysis of the cells. This is reflected in the extremely short D_2 value. There are several possible sites on, and within the cell, which could react with the plasma, ultimately leading to cell death".

Khomich et al. (1997) report on sterilization by the glow of a d.c. discharge (direct exposure to plasma) at pressures in the range 0.05–0.2 Torr. They show that the charged particles (ions, electrons) of the plasma glow do not play an essential role in inactivating microorganisms. In the glow of a d.c. discharge, ion energy does not exceed a few eVs. In contrast, Lisovskiy et al. (2000) show that in a capacitive (parallel plate) RF discharge, operated at RF voltages such that the ions reach energies between 100 and 200 eVs, ion bombardment of the microorganisms plays a major role in their inactivation.

Khomich et al. (1998) in a more recent experiment and then Soloshenko et al. (1999) claimed to have isolated the action of neutral particles on sterilization from that of UV radiation, concluding that the inactivation time by neutral particles only is merely twice longer in an oxygen discharge and from five to six times longer in an air discharge, as can be seen from Fig. 4. Actually, it is

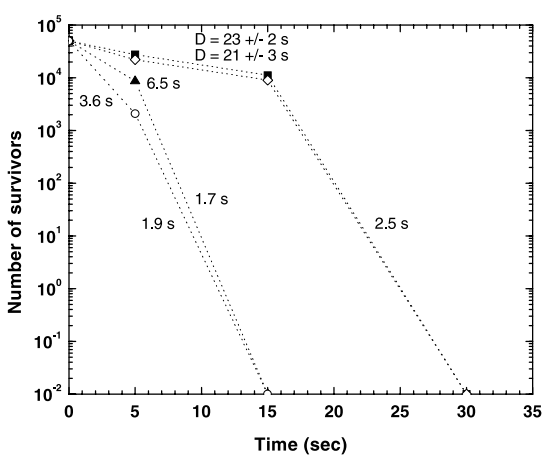

Fig. 3. Survival curve of *S. aureus* and *E. Coli* cells (5.0×10^4) seeded on polypropylene samples and exposed to a DBD-type discharge in air at atmospheric pressure: *S. aureus* cells exposed unwrapped (◇) and in semi-permeable bags (■), *E. coli* cells (○) exposed unwrapped and in semi-permeable bags (▲; after Kelly-Wintenberg et al., 1998).

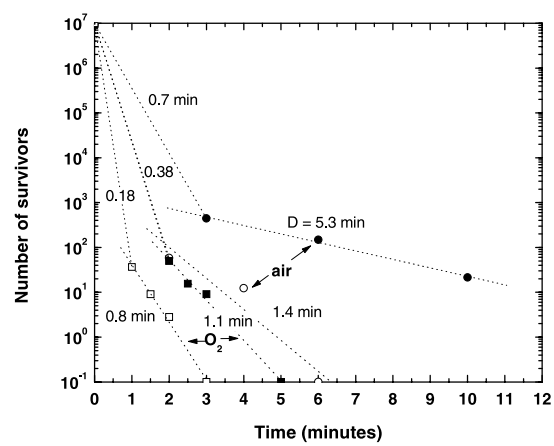

Fig. 4. Survival curves for *B. subtilis* spores exposed to UV radiation only (open symbols) and to neutral reactive particles only (closed symbols) originating from a d.c. discharge at 0.2 Torr (after Soloshenko et al., 1999).

quite possible that excited neutrals (which could go through their deflector) provided UV photons within the sterilization chamber. These authors believe that the sterilization of complex form objects (eg cavities) actually needs to rely on neutral particles because the action of UV photons is limited by shadowing (Soloshenko et al., 2000). They further show (Soloshenko et al., 1999) that the most efficient sterilization precursor is O_2 followed, in decreasing order, by air, CO_2, H_2, argon and N_2. They observe that sterilization time is practically independent of gas pressure for all the above gases in the range investigated, but it decreases when increasing the discharge power density. Their sterilization time is short, typically 3 min for an initial population of 10^7 *B. subtilis* spores (spore suspension deposited on Petri dishes of 10 cm^2 surface) in an oxygen discharge.

Fig. 4, from Soloshenko et al. (1999), shows that a plasma sterilizer survival curve is not made from a unique straight line, but comprises two or three segments of straight lines, as already noted in Figs. 2 and 3; complete inactivation (at least in the medium and low-pressure range) seems to imply a three-phase process, as it was underlined in relation with Fig. 2 (a low-pressure case compared to the present medium-pressure situation). These slopes follow the same trends as in Fig. 2: the first step is the fastest (smallest *D* value) and the second one is the slowest (largest *D* value). Fig. 5, also from Soloshenko et al. (1999), compares plasma and mercury lamp sterilization (sterilization with the Hg lamp is achieved in ambient air). An important point to remember for further consideration is that the Hg-lamp inactivation diagram exhibits a two-step process, the first one having the smallest *D* value. A similar survival curve is obtained with an UV (248 nm) excimer laser (Warriner et al., 2000).

Moreau et al. (2000) used the flowing afterglow from a microwave discharge sustained in pure argon and pure oxygen, in mixtures of argon and oxygen and mixtures of nitrogen and oxygen. The gas pressure in the sterilization chamber was usually in the 2–7 Torr range and flow rate in the range of 0.5–2 standard liters per minute. The test spores were *B. subtilis*, deposited on the bottom of stainless steel receptacles of approx. 10 mm diameter (exposure surface of approx. 1 cm^2). Total inactivation of an initial 10^6 spore population was obtained with $2\%O_2/98\%N_2$ in approx. 40 min, a much longer time than with direct exposure to plasma.

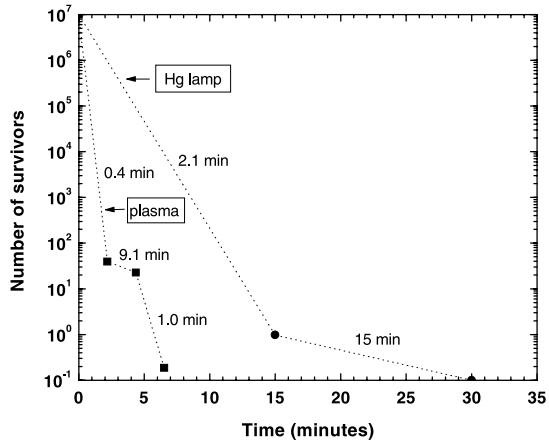

Fig. 5. Survival curves for *B. subtilis* spores exposed to UV radiation from two different sources: (■) an air plasma (power density—0.1 mW/cm^2; ●): a mercury lamp (power density—1.5 mW/cm^2; after Soloshenko et al., 1999).

Fig. 6 compares the sterilization efficacy of a pure argon afterglow with that of an argon mix-

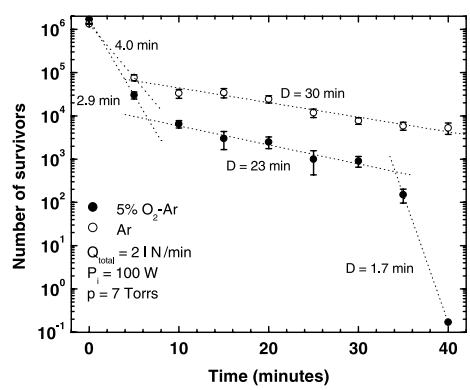

Fig. 6. Survival curves of *B. subtilis* spores subjected to a flowing afterglow. Adding molecular oxygen to argon gas in the discharge leads to complete inactivation of the spores in 40 min (Moreau et al., 2000).

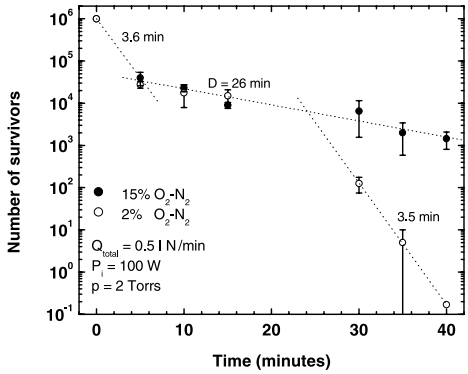

Fig. 7. Survival curves of *B. subtilis* spores subjected to a flowing afterglow in two O_2/N_2 mixtures: the 15% O_2 mixture yields the highest oxygen-atom concentration while the 2% O_2 mixture maximizes the NO_β UV emission (Moreau et al., 2000).

ture containing 5%O_2. Complete inactivation[1] is readily obtained, in three steps, in the presence of O_2 in the discharge. Moreau et al. (2000) suggest that the (partial) killing of endospores in the pure argon afterglow results from the action of UV photons; the third step, specific to the oxygen/argon mixture, is clearly related to the presence of oxygen atoms. The addition of O_2 to Ar decreases the value of D_2 by approx. 25%.

Fig. 7 is the result of an optimization process. The O_2 percentage content in the nitrogen-based mixture yielding the largest oxygen-atom concentration [O] was determined through a titration method (Ricard et al., 2001); under the present operating conditions, [O] was found to first increase with increasing O_2 percentage in the mixture and then reach a maximum value and saturate at O_2 percentages higher than 12%, hence its setting at 15% in the first part of the experiment. In contrast, the maximum intensity of UV radiation connected with the excited NO molecules formed in the afterglow is maximum slightly below 2% O_2 in the mixture. Fig. 7 clearly shows that the maximum of inactivation efficacy is achieved when the UV emission intensity is maximum. Nonetheless, the presence of O atoms is essential and one can speak of a synergistic effect between UV photons and O atoms (Moisan et al., 2001b; Philip et al., 2001).

Finally, Fig. 8 shows the influence of the sample holder temperature on the inactivation mechanism. When the substrate is at 50 °C compared to 15 °C, the first phase eliminates more bacteria and the third phase shows up, suggesting a thermally enhanced process.

Moreau et al. (2000) underline that a critical point of the flowing afterglow system is the gas flow conditions (flow regime and flow rate), which should be such that all parts of the objects to be sterilized get in contact with the active species of the afterglow.

Lerouge et al. (2000c) used direct exposure of *B. subtilis* endospores to a 2.45 GHz microwave discharge sustained in various gases (O_2, CO_2, O_2/Ar, O_2/H_2, $O_2/H_2/Ar$ and O_2/CF_4) in the medium-pressure range (80 mTorr). To keep the gas temperature low, the discharge was applied in the form of 30-s pulses followed by 30-s pauses. Complete inactivation of the spores generated three-phase survival curves, as discussed above, except for the O_2/CF_4 gas mixture where only the first and second phases are present.

Lerouge et al. (2000c) aimed at showing that plasma sterilization is akin to synthetic polymer etching. Fig. 9 presents photographs from scanning

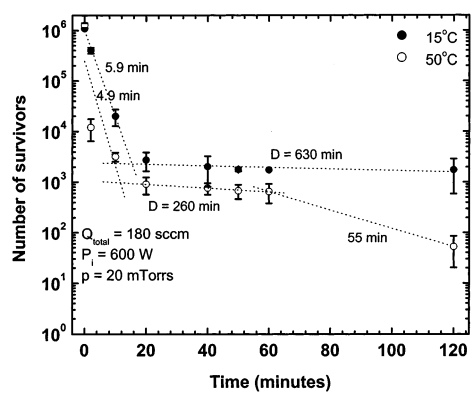

Fig. 8. Survival curves of *B. subtilis* spores subjected to the flowing afterglow of a pure O_2 discharge, showing the influence of the substrate temperature on the sterilization efficacy (adapted from Hury et al., 1998).

[1] When making use of survival curves, since zero detected spore cannot be represented on a semi-log scale, one usually plots the corresponding result in terms of an estimated probability of finding no spores. In the present case, sterility is observed at 40 min and it is plotted as 1/6, because no spores were found over the six independent experiments performed.

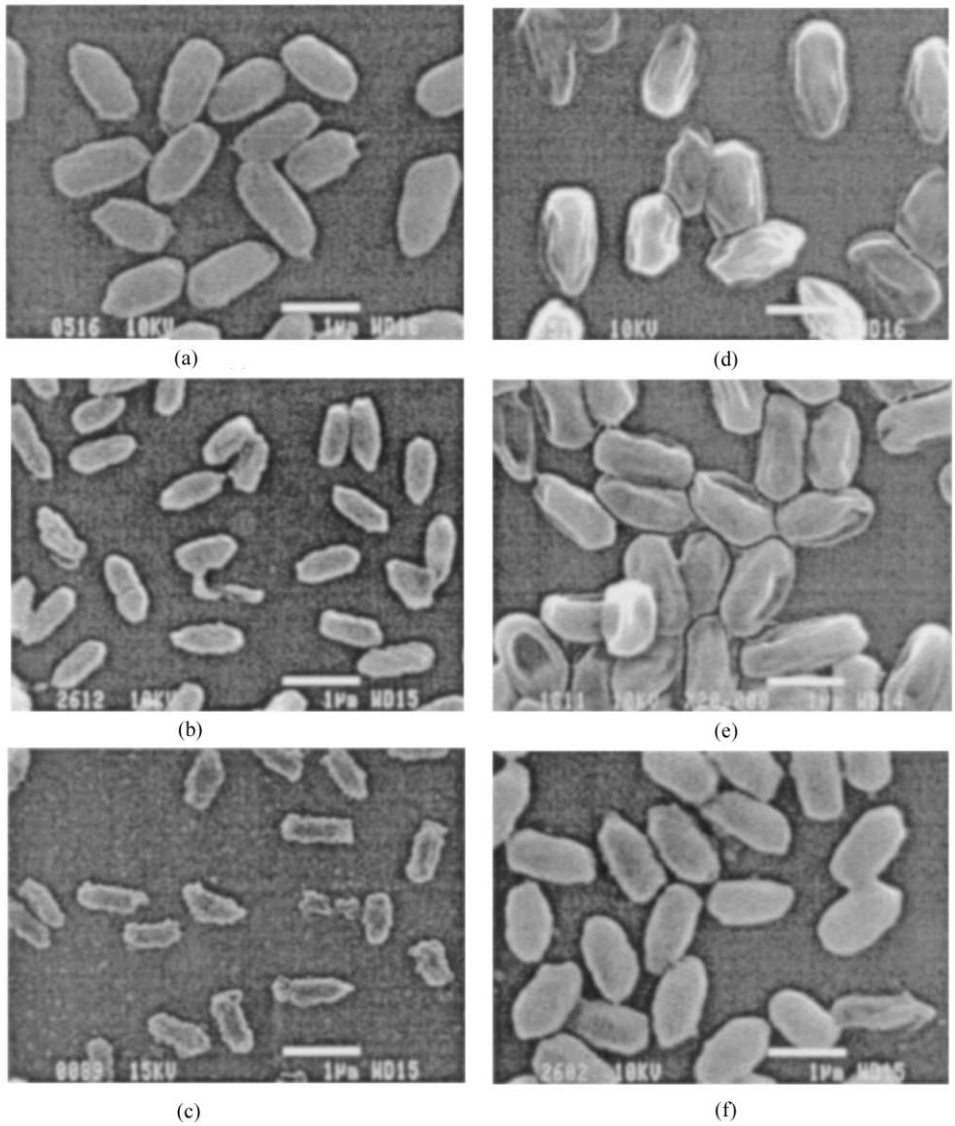

Fig. 9. Scanning electron micrographs of *B. subtilis* spores: (a) untreated control; (b) spores exposed 15 min to a pure O_2 plasma; (c) spores exposed 15 min to a O_2/CF_4 plasma (80 mTorr, 200 W; 70 sccm; $[CF_4] = 15\%$); (d) spores treated by Sterrad-100S®; (e) spores treated by steam autoclaving (20 min at 121 °C); and (f) spores treated by pure EtO (Lerouge et al., 2000c, reprinted by permission of Wiley, New York).

electron microscopy (SEM) of spores after various treatments. Spores exposed for 15 min directly to a pure O_2 plasma (Fig. 9b) exhibit a significant reduction of their initial dimensions, in clear contrast to the case of spores inactivated by conventional sterilization means (Sterrad®, autoclave and EtO: Fig. 9d, e and f, respectively) where there is no shrinkage. Laroussi et al. (2000) have observed a stronger erosion process when exposing *E. coli* to a glow (DBD-type) discharge at atmospheric pressure.

Lerouge et al. (2000b) have also examined the role of UV and VUV radiation on the killing of *B. subtilis* endospores, showing the importance of the radiation wavelength in the inactivation process.

3.5. The case of plasma-based sterilization systems

In some commercial systems, termed plasma sterilizers (eg Sterrad® and Plazlyte®), the plasma has no biocidal action, but serves simply as a detoxifying agent, removing noxious residues and limiting the oxidation effect of the highly reactive chemical elements (hydrogen peroxide and peracetic acid-based mixture, respectively) that are injected in the form of vapors as the sterilizing agent (Krebs et al., 1998). The survival curve of such systems exhibits a unique straight line (Cariou-Travers and Darbord, 2001), as with conventional sterilizers, in contrast to true plasma sterilizers. As a matter of fact, these systems, although efficient, are not plasma sterilizers according to our definition (Section 1), which states that the gas or gases used have no biocidal effect unless they are activated by an electrical discharge.

3.5.1. Basic principle of plasma-based sterilizers

Living microorganisms are hygroscopic, which means that water vapor in the ambient gaseous phase condenses preferentially on their surfaces. In a similar way, peroxide vapors like hydrogen peroxide and peracetic acid, or oxidative gases like ozone, can condense on nucleation sites such as those formed by bacteria lying on a smooth supporting surface, provided the ambient conditions (pressure and temperature) are appropriate. Viruses, like any surface heterogeneity with dimensions exceeding the critical nucleation diameter (which is approx. 1 nm), are also efficient nucleation sites (Marcos-Martin et al., 1996). This condensation mechanism is the basic principle of the plasma-based, commercial sterilizers that go through a chemical-gas cycle before the glow discharge is used.

3.5.2. Description of two commercial plasma-based sterilizers

These are the hydrogen peroxide plasma-based (HPpb: Sterrad®) sterilizer (Jacobs and Lin, 1987) and the peracetic acid plasma-based (PApb: Plazlyte®) sterilizer (Campbell and Moulton, 1995). In the HPpb case, the chemical phase is provided by 1.8 ml of hydrogen peroxide at a concentration of 58% vol., while in the PApb system, it comes from a mixture of peracetic acid (5%), hydrogen peroxide (22%), acetic acid (10%) and water (63%). In both cases, the chemical phase is followed, on each sterilization cycle, by a plasma phase, as shown in Figs. 10 and 11.

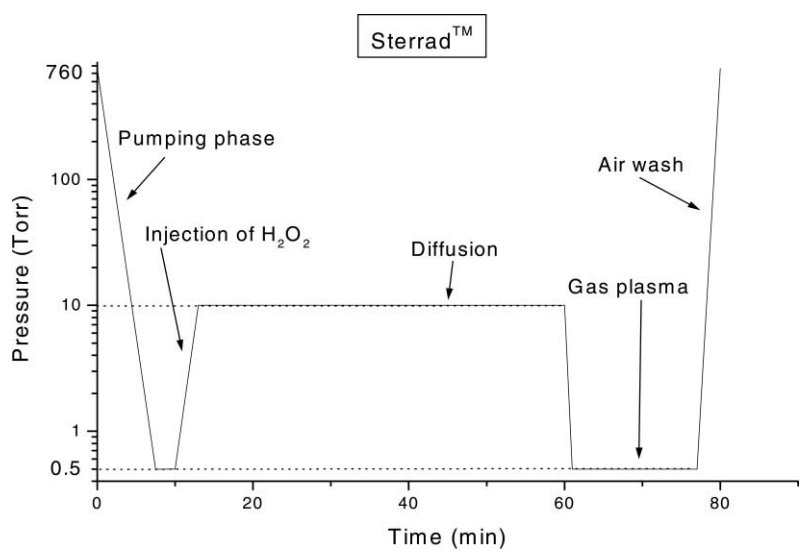

Fig. 10. Operating time-sequences of the Sterrad 100 plasma-based sterilizer (after Jacobs and Kowatsch, 1993).

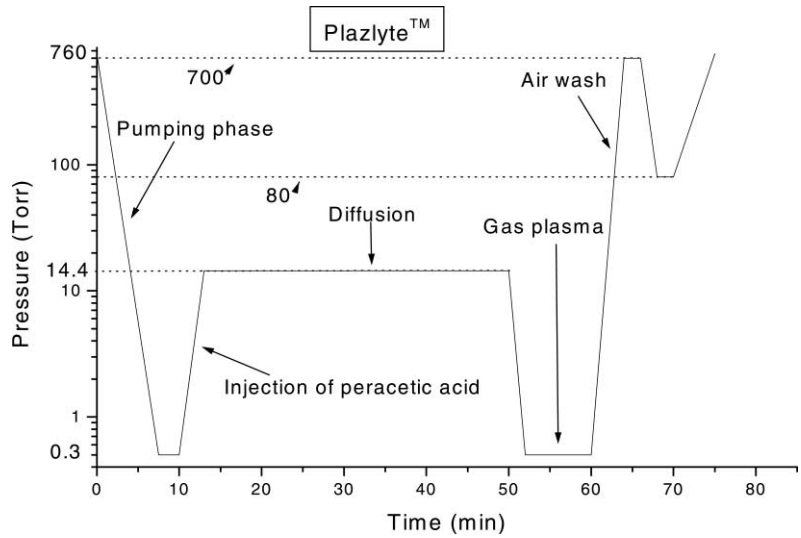

Fig. 11. Operating time-sequences of the Plazlyte plasma-based sterilizer (after Crow and Smith, 1995).

The first step of the HPpb process consists in achieving a moderate vacuum (500 mTorr/65 Pa) in the sterilization chamber. Then, hydrogen peroxide is injected into the sterilizer at a temperature of 45 °C, reaching a vapor pressure of 10 Torrs (1300 Pa). The initial low base-pressure ensures an efficient diffusion of the vapor through the packaging material towards the objects to be sterilized. The condensation of this vapor on the nucleation sites offered by the microorganisms leads to their inactivation. After this step, a radio-frequency electromagnetic field is switched on to create a glow discharge at the periphery of the chamber; this part of the process lasts from 16 to 19% of the total cycle, depending on the degree of moisture in the samples being treated. The final stage is the slow return of the chamber to the atmospheric pressure (air wash). Variants of the above procedure have been recently examined (Cariou-Travers and Darbord, 2001). Fig. 11 shows the cycling procedure for the PApb system. Instead of achieving the detoxifying discharge in the sterilant vapor as in the HPpb case, a plasma is produced in a mixture of oxygen, hydrogen and argon, which is flown out of the discharge region to achieve an afterglow. The entire cycle operates at a temperature of 50 °C for both apparatuses and takes 75 min in the case of HPpb and 60 min with PApb.

In both systems, it is the condensation of the chemical vapor phase on the microorganisms that provides the sterilizing action (Krebs et al., 1998). This is why, in our opinion, these systems are unduly referred to as plasma sterilizers, which does not change the fact that they can achieve complete sterilization of biomaterials, as reported by many studies (Jacobs and Kowatsch, 1993; Crow and Smith, 1995; Kyi et al., 1995; Alfa et

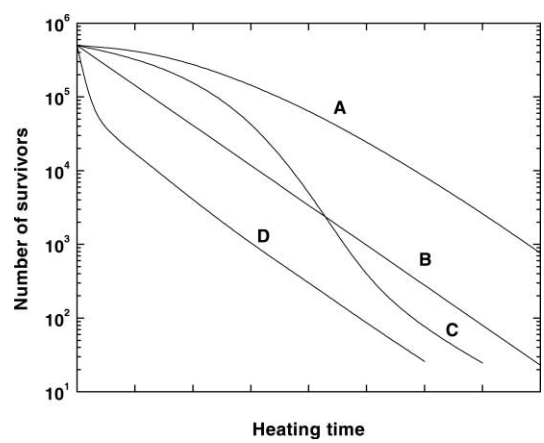

Fig. 12. Classical survival curve (B) and three other commonly observed non-exponential survival curves (A, C, and D) designated as convex, sigmoid and concave curves, respectively, resulting from thermal inactivation (after Moats, 1971).

al., 1996; Borneff-Lipp et al., 1997; Vassal et al., 1998; Vickery et al., 1999).

4. Analysis of the survival curves and suggested mechanisms of plasma sterilization

The rate of inactivation of microorganisms exposed to a given stress is most generally exponential and, as such, is adequately reported by their survival curve. We examine such plots for bacterial endospores since they are the most resistant microbial form to environmental stresses including heat, UV radiation and chemicals. Our study is limited to the case of a homogeneous population of spores.

4.1. Summary of the mechanisms of classical sterilization

Lethal heat energy achieved by wet heat (autoclaves) or dry heat (ovens) leads to the ultimate death of all living forms by destroying the cellular metabolic system, which includes enzymatic components. Wet-heat systems require a water content sufficient to produce 100% relative humidity at the sterilization temperature, otherwise it is dry-heat sterilization.

The high hydrostatic pressure exerted in autoclaves is believed to induce the germination of spores and then to kill the germinated cells. Such a wet-heat process induces several changes in the cell: leakage of low-molecular-weight material through breaches made in the cell wall, RNA and DNA breakdown, and protein coagulation. Inactivation of microorganisms by dry heat may be considered primarily an oxidation process.

Fig. 12 shows a classical survival curve with its unique straight line and three other commonly observed forms, all resulting from thermal inactivation. According to Pflug and Holcomb (1991), curve B corresponds to approximately one-third of the experimentally determined survival curves for homogeneous cultures of microorganisms exposed to identical (heat) stress conditions. Curves A and D in Fig. 12 probably constitute another one-third of the results: during the initial heating time period, the death rate is lower or higher than the major straight-line portion. Curve C is similar to curve A, but tails probably because of a small population of heat-resistant cells. Concave curves similar to curve D are generally interpreted as indicating a heterogeneous population with regard to heat resistance (Moats, 1971).

Chemical sterilization is essentially a process where an item is immersed in the sterilant or exposed to it in a vapor state (chemiclaves, ethylene oxide sterilizers). The corresponding most widely used sterilants are glutaraldehyde, hydrogen peroxide and ethylene oxide. The biocidal activity of ethylene oxyde is due to alkylation of different chemical groups in the spore or the vegetative cell. The biocidal effect of glutaraldehyde resides in its interaction with outer cell-layer proteins and glycoproteins, and with the inhibition of RNA, DNA and protein synthesis. Hydrogen peroxide is a strong oxidant and, at high concentrations (10–30%), it is biocidal; the hydroxyl radical produced during its action is believed to do the actual killing of microorganisms by attacking the membrane lipids, DNA and other essential constituents (Morris, 1970). It generally yields straight-line survival curves such as curve B in Fig. 12 (Block, 1991).

4.2. The active species and the mechanisms involved in plasma sterilization

Plasma sterilization implies completely different mechanisms than those of classical sterilization techniques. Our interpretation of the survival curves assumes the three following basic processes:

1. Direct destruction by *UV irradiation* of the genetic material of the microorganism.
2. 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 non-equilibrium chemistry are small molecules (eg CO and CH_x should then be possible).

3. Erosion of the microorganism, atom by atom, through *etching*. Etching results 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 OH, respectively, and excited molecules, for example, the $^1O_2$ singlet state. This chemistry, under thermodynamic equilibrium conditions (Section 3.2), yields small molecules (eg CO_2, H_2O), which are the final products of the oxidation process. 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. This UV-induced chemistry under non-equilibrium conditions can result in the desorption of radicals and molecules, at both the intermediate and final stages of oxidation.

In both cases 2 and 3, the by-products are removed through pumping (or gas flushing) and the gas phase is replenished with 'new' reactive species.

4.2.1. The active species

The presence of specific reactive particles and UV emitters in the (glow or afterglow) gas phase depends strongly on the operating pressure. For example, ozone is more readily observed essentially at atmospheric pressure. UV photons at pressures as high as atmospheric pressure can be strongly reabsorbed in their generating plasma, preventing them from reaching the samples to be sterilized. This is the case, in particular, of vacuum UV (VUV) photons ($\leqslant 180$ nm). This is why UV photons generated in the glow or afterglow are expected to be most efficient in the medium and low-pressure ranges.

The efficiency of UV photons in the sterilization process depends on the basic mechanism or mechanisms activated at the wavelength considered, namely, (A) the inactivation of the genetic material by UV radiation; (B) and (C), the erosion atom by atom of the microorganism material. It also depends on the intensity of this UV emission: Fig. 7 tells us that below some minimum UV intensity, etching is not efficient, a point only recently demonstrated. Understanding the role of photons is further complicated by reports claiming the possibility of achieving sterilization with neutrals only (Fig. 4). All these facts and the limited penetration depth of UV photons (which necessitates distinguishing between isolated and stacked spores) are at the origin of the controversy concerning the respective roles of UV radiation and reactive neutral species. The explanations provided below with reference to the survival curves yield a clearer and more coherent picture on this matter.

Besides atomic oxygen and ozone, other active species such as O_2 in the metastable singlet state can be present in the gas phase of oxygen-based discharges. More generally, particles in a metastable energy state can be found in any discharge. Such excited species have a very long lifetime (sometimes of the order of seconds or longer) since they cannot emit photons through an electric-dipole transition, a very fast ($\approx 10^{-8}$ s) de-excitation process. Under most experimental conditions, however, the metastable species de-excite through collisions prior to emitting a photon, transferring their energy to other particles, eventually activating chemical reactions in the gas phase of the plasma or on surfaces exposed to it. These metastable species, therefore, act as a reservoir of energy for plasma chemistry.

4.2.2. The various phases on the survival curve: tentative explanations

The proposed line of explanations in the case of a three-phase survival curve (eg Fig. 7) assumes the following: (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) spores are generally inactivated by UV photon irradiation of their DNA material (mechanism A). From these hypotheses, the observed phases are attributed the following roles:

1. The first step mainly reflects the kinetics of DNA destruction by direct UV irradiation of the isolated (meaning non stacked) spores and of the very first layers of them on a stack. It provides the highest killing rate (smallest D value), but it cannot lead to sterilization because of the limited penetration depth of UV photons into stacked spores or spores covered with various debris.
2. The second step, which has the slowest kinetics (largest D value), reflects the time required for sufficient (partial) erosion by mechanisms B and C of inactivated spores and debris of all kinds standing on top of living spores.
3. The third step only comes fully into action when the last living spores have all been sufficiently cleared from debris for the UV photons to directly hit their genetic material. 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.

The third step starts only after some delay with respect to the beginning of the second phase. This delay is the shortest when neutral reactive species such as O-atoms are present and when the UV emission intensity has been maximized (Moreau et al., 2000; Philip et al., 2001): such optimized conditions correspond to UV-induced etching (mechanism C enhanced by UV photons). In the presence of O-atoms, but with a non optimized photon flux, spontaneous etching can eventually dominate over photodesoprtion; phase 3 is reached but after a longer time than when the UV photon intensity is maximized. In the absence of reactive species (eg O-atoms), only intrinsic photodesorption (mechanism B) can take place, hence a much longer delay time before eventually reaching sterilization (case of Hg lamp in Fig. 5). The action of UV photons alone give rise to two-step survival curves (Figs. 4 and 5). We now consider the arguments in favor of our interpretation.

4.2.3. Experimental facts in support of the interpretation of the first phase

As stated above, this step is assumed to correspond to the inactivation of isolated spores, and of top ones in the case of stacked spores. According to this reasoning, the more isolated spores there are, the larger number of them should be inactivated during this first phase, which is what Fig. 2 shows: a three times larger exposure area, resulting from a larger dilution of the suspension actually yields one more decade of inactivated spores.

The fact that UV photons are the main contributors to spore inactivation in this first step can be inferred from noting that the first slope of the survival curve is also the steepest one in a pure argon afterglow (Fig. 6), where it can be expected that only UV photons are the active species. The dominating role of UV photons in this first phase is further confirmed by the survival curves of spores exposed to 254 and 248 nm UV photons from a Hg lamp (Fig. 5) and an excimer laser (Warriner et al., 2000), respectively: there are two slopes and the first one is also the steepest.

Such a first phase dominated by UV radiation seems to exist whatever the nature and composition of the gas, as shown in Figs. 4–8, provided the discharge is operated in the low or medium-pressure range where photon absorption by the carrier gas is not too high. In contrast, at atmospheric pressure, the initial slope of the observed biphasic curve (Fig. 3) is not the steepest one, which could stem from the fact, as mentioned by Kelly-Wintenberg et al. (1998), 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 'get out' and reach the top spores.

4.2.4. Experimental facts in support of the interpretation of the second phase

Our assumption is that the inactivation kinetics of this second phase is limited by erosion processes (mechanisms B and C). Intrinsic photodesorption (mechanism B) is the only possible erosion mechanism with Hg lamps or UV lasers in ambient air (Fig. 5). Comparing the D_2 times in Fig. 6, we observe that it is 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). Fur-

thermore, the D_2 time is shorter when the substrate temperature T is increased, as shown in Fig. 8, which implies that mechanism C is then present. Finally, the reduction in the spore's size observed by Laroussi et al. (2000) and Lerouge et al. (2000b); Fig. 9) is a direct proof of the existence of an erosion mechanism.

4.2.5. Experimental facts in support of the interpretation of the third phase

This phase begins when only a small fraction of the initial number of spores remains to be inactivated. It starts relatively rapidly when there are oxygen atoms and a high flux of UV, otherwise it can be delayed a few hours (Philip et al., 2001). As can be seen from Figs. 6 and 7, the D_3 time value can be close to that of D_1, which then supports the assumption of DNA inactivation by direct UV irradiation in this third phase inactivation. However, sometimes D_3 is much larger than D_1 (Fig. 8) and the inactivation kinetics could then rely on both the erosion and the DNA destruction.

5. Conclusion

In the pure form of plasma sterilization, the gas or gases involved have no biocidal effect unless they are activated by the electric field of the discharge. The corresponding active species are present only when the discharge is on and disappear some milliseconds after the discharge has been turned off, which means that no vent time is required.

True plasma inactivation (in contrast to plasma-based techniques) is characterized by the existence of two or three distinct phases in the survival curve of a homogeneous population of spores. These phases correspond to changes in the dominating kinetics of the inactivation process as a function of exposure time. In the case of medium and low-pressure discharges, the inactivation mechanisms are the DNA destruction by UV irradiation and the 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 abnormal prions, which have no genetic materials, could a priori be achieved through the erosion of these proteins. In this case, etching enhanced by UV radiation would be the fastest erosion mechanism. The operating conditions would then correspond to those for the shortest sterilization time of microorganisms, but the required time to inactivate prions might be longer because it could necessitate a higher degree of erosion.

The controversy concerning the respective roles of UV photons and reactive radicals has been resolved in the case of reduced-pressure plasma sterilization. A synergistic action of UV photons and O atoms is actually required to minimize the sterilization time; in the case of the N_2–O_2 mixture, the percentage of O_2 should be set such that the UV radiation from the NO_β band is maximized.

The use of UV lamps or lasers in ambient air instead of plasma restricts the erosion of microorganisms to photodesoprtion, a less efficient mechanism than etching. It also leads to shadowing effects (which includes the difficulty to sterilize crevices), in contrast to gas plasma sterilization where the UV photon is brought to the appropriate site by its emitting atom or molecule.

Direct exposure to plasma yields shorter sterilization time than exposure to the plasma afterglow, the latter being generally safer, easier and less expensive to operate. Of the three pressure ranges considered, the low-pressure (mTorr) regime yields the longest sterilization time because of its lowest concentration in neutral active species.

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 their DNA material. It implies that any material covering microorganisms increases the time required to achieve sterilization since erosion, a slow process, has to come into play. In the future, reports on plasma sterilization should specify both the initial number of spores and the surface covered by the suspension, and include some indications as to the organic load or 'cleanliness' of the suspension.

Further research must establish the degree of damage to surfaces of different materials subjected to the discharge glow or afterglow. There also remains to determine the efficacy of sterilization of small diameter, long cylinders such as endoscopes and the ability to sterilize devices in their packaging.

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