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Sterilization by Low-Pressure Plasma: The Role of Vacuum-Ultraviolet Radiation

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Low-pressure plasma is a promising method for destroying microorganisms, an alternative to "conventional" methods, which have numerous drawbacks. Several plasma-based sterilization technologies are presently under development, even though the exact role of the various plasma constituents, for example ultraviolet radiation, on the sterilization mechanism is still unknown and subject to controversy. In this study, we first report high sporicidal activity of a microwave (MW) plasma compared to its radio-frequency (RF) counterpart, which we believe to be due to the higher concentration of reactive particles in the former plasma. We then report a relatively low sporicidal efficacy of vacuum ultraviolet (VUV) radiation (between 115 and 170 nm) emitted by an hydrogen MW plasma, in spite of the high effectiveness of these photons to break chemical bonds. We discuss these results in terms of etching (ablation), which we have observed for both synthetic polymers and spores, and in terms of other possible mechanisms proposed in the literature. The sporicidal effectiveness of VUV/UV radiation appears to vary markedly with wavelength and intensity, on account of spore structure and molecular absorption.

KEY WORDS: Sterilization mechanism; low-pressure plasma; vacuum-ultraviolet; bacterial spores; etching.

1. INTRODUCTION

Plasma sterilization is a relatively new application of low-pressure plasma. It is a promising alternative to other sterilization methods, such as those

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based on elevated temperature (steam autoclave and dry heat), gamma-irradiation and ethylene oxide, which are generally not well adapted to the new challenges of clinical sterilization (short sterilization cycle, low temperature, absence of toxic residues, etc.).

A low-pressure plasma can be briefly defined as a partially ionized gas, comprising charged particles (ions, electrons), neutral particles (atoms, excited and ground-state molecules, radicals) and photons covering a broad spectral range; the latter mainly originate from deexcitation of various excited species present. Plasma is generally created by applying high frequency (e.g., radio-frequency (RF) or microwave (MW)) electric power to the gas; a key reason for using plasma in sterilization, is the presence of the highly reactive species (more reactive than "conventional" chemical species), while maintaining items to be sterilized (e.g. polymers) near ambient temperature.

Oxygen-containing plasmas have been shown to be capable of destroying microorganisms, but the mechanism is still unclear: the roles of the various types of reactive particles are not known and subject to controversy.[1–7] It has been suggested that ions and electrons play a minor role in plasma sterilization, while ultraviolet radiation and reactive neutral particles (such as atomic oxygen) are thought to be the principal sterilizing agents of the plasma. However it is still unclear whether radicals or photons play the main role in the destruction of microorganisms in plasmas; yet, a better understanding is necessary to develop more efficient sterilization systems.

For the study, development, and validation of sterilization processes, one generally uses bacterial spores, which are the most resistant form of living microorganisms. Spores are produced by differentiation of vegetative bacteria after exhaustion of necessary nutrients, during which a number of new morphological structures are formed, including proteinous outer layers, which contribute to their extraordinary resistance to sterilization processes.[8]

The effect of plasma and of UV radiation on polymers is an important research topic, including at Ecole Polytechnique.[9–15] Since microorganisms bear certain similarity to synthetic polymers, being macromolecules composed of the elements C, H, N and O, our approach has been to draw parallels between the destruction of microorganisms and the abundant literature on plasma treatment and etching of polymers. In previous articles, we have shown that a mortality rate of *Bacillus subtilis* spores of more than 5 log in 5 minutes can be achieved with a MW O_2/CF_4 plasma (88/12%).[6,7] This particular gas mixture was found to exhibit a much higher efficacy than all other gases or gas mixtures that we have tested, an observation we have correlated with its high etching rate.[9–11] Thus, etching appears to be a key mechanism in plasma sterilization, but many associated issues remain unclear. We believe that the high efficacy of our O_2/CF_4 plasma system, compared to other published results[3,5,16,17] is due to the gas composition and the high

flow rate, as further explained in the previously mentioned publications,[6,7] but also to our choice of a MW power source: In contrast to other systems like Sterrad®, which use RF (13.56 MHz) excitation,[18] we used MW (2.45 GHz) power. Differences between MW and RF plasma, the object of in-depth earlier investigation in these laboratories,[19,20] are attributed to different electron energy distribution functions and to a resulting higher population density of energetic electrons in the MW case, which gives rise to higher concentrations of reactive precursors.[19,21] An aim of our study was therefore to compare RF and MW plasma in terms of bactericidal activity.

A second objective of this work has been to study the effect of plasma UV/VUV radiation on microorganisms. As already mentioned, plasmas emit electromagnetic radiation ranging from far ultraviolet to the infrared. Figure 1 presents the terms employed in this paper for the ultraviolet part of the spectrum, subdivided into several wavelength (or energy) ranges: (1) ultraviolet (UV), from 200 to 380 nm, and (2) vacuum-ultraviolet (VUV), below 200 nm, so called because it is absorbed in atmospheric oxygen. The UV portion, in turn, can be separated into UV-A, UV-B and UV-C, which have been subject to numerous studies in photobiology, for example for its mutagenic and mortality effects on cells through DNA damage. UV-C at 254 nm has been used for many years for surface disinfection, especially in the food industry, and for the prevention of infections in healthcare facilities, but the efficacy of this procedure is often questioned.[22–25]

Far-UV ($100 < \lambda < 200$ nm) is an important component of VUV emission from plasma, since these photons have energy which greatly exceeds that of all chemical bonds in organic molecules, and thus may attack microorganisms by breaking bonds in their protective membranes.[14,26,27] However, the role of VUV/UV radiation from plasma is also a subject of some controversy: according to some authors, UV radiation plays the main role in plasma sterilization of open surfaces,[28] while other authors claim its effect to be negligible.[1]

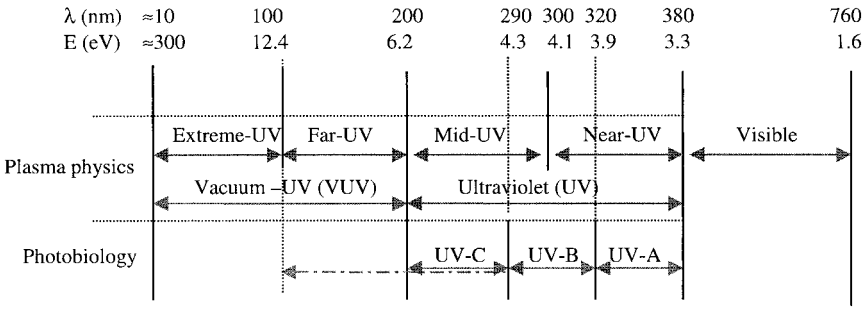

Fig. 1. Wavelength and energy of radiation in the ultraviolet and visible portions of the spectrum, as used in plasma physics and in photobiology.

Extensive recent studies in our laboratory,[12–15] have examined VUV/UV emissions from various plasmas, and their effects on different polymers, for example etching: while VUV radiation alone can etch (or ablate) polymers, synergistic interaction of VUV and reactive oxygen species (particularly atomic oxygen-AO) created by VUV radiation in an oxygen atmosphere gives rise to higher etch rates; AO alone was found to have only little effect.[13] Since we have shown that etching is a key mechanism of spore destruction by plasma,[6,7] a second objective of the present work has been to study the effect of VUV/UV radiation on spore mortality, for example in the form of possible synergies similar to those observed for polymer etching.

2. EXPERIMENTAL METHODOLOGY

2.1. Sterilization Experiments with MW and RF Plasma

Bacillus subtilis (Bs) spores (ATCC 9372 : lot 973087, Spordex®, AMSCO, Erie, PA), known as the most resistant type of spore to plasma, were used in this study. As described in our previous paper, one hundred microliters of Bs suspension ($N_0 = 10^7$ spores) was aseptically spread on the flat bottom of shallow borosilicate glass vials ($\Phi = 25$ mm, $h = 18$ mm) made specially for this study, in the form of a monolayer, and left to dry.[6,7]

Plasma sterilization treatments were carried out in a "large-volume microwave plasma" (LMP™) reactor chamber, described elsewhere.[7,9] The experimental set-up for MW and RF plasma experiments is shown schematically in Fig. 2. In the first case, MW power (2.45 GHz) was applied from a strapped-bar slow-wave structure through a rectangular fused silica window into the stainless steel reactor chamber. The grounded stainless steel sample holder was maintained parallel to the window, so that the open ends of the vials were immersed in the glow zone. In a second set of experiments, RF power (13.56 MHz) was used instead, as shown on Fig. 2b; to study the effect of ion bombardment, vials were mounted on the RF-powered electrode, where a d.c. bias potential, $V_b = -150$ V, was maintained. For comparison, nominally identical sets of experiments were carried out, during which vials were mounted on the grounded electrode, thereby being exposed to a flux of lower-energy ions.[11] Oxygen/CF_4 (88%/12%) gas mixture was used for this investigation, based on our previous results.[6,7] Except for the excitation frequency, other experimental parameters were nominally identical: Total applied power was maintained at 200 W (see later description), total gas flow rate at 80 standard cubic centimeters per minute (sccm), and the operating pressure at 80 millitorr (11 Pa). In each case, three vials were exposed to the plasma for 5 minutes. The effect of pure O_2 MW plasma was also determined, for reference and comparison with our earlier results.[6,7]

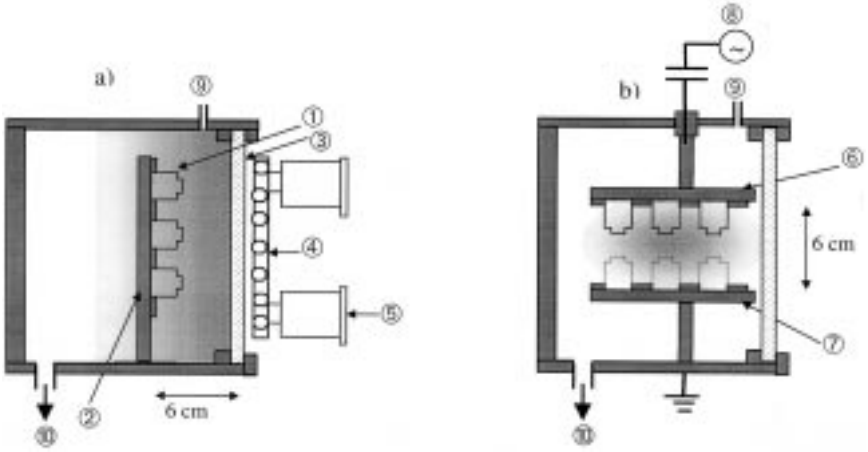

Fig. 2. Schematic representations of the plasma system used for (a) MW and (b) RF plasma treatments. In this latter configuration, samples were placed either on the powered electrode ($V_b = -150$ V) or on the grounded electrode ($V_b = 0$): ① glass vials inoculated with Bs spores; ② sample holder; ③ silica window; ④ microwave applicator; ⑤ microwave generator; ⑥ RF-powered electrode; ⑦ grounded electrode; ⑧ RF (13.56 MHz) power supply; ⑨ gas inlet; ⑩ to vacuum pump.

After treatments, the spores were recovered aseptically in brain heart infusion medium, serially diluted, spread on blood agars, and incubated during 24 hours at 37°C. For each sample ($n = 3$), the number of viable spores after exposure to plasma (N) was determined by averaging the number of regenerated colonies counted on 3 agar plates. Then, the log reduction of spore count, also called mortality (M), was calculated by averaging log (N_0/N) for given treatment conditions, where N_0 is the initial number of viable spores, calculated from 3 control vials:

$$M = 1/3 \sum_{3} \log (N_0/N) \tag{1}$$

where each value of N is, in turn, the average of three determinations.

2.2. Sterilization Experiments with VUV Radiation

To study the effect of VUV radiation on spores, we have used a second "*in-house*" experimental system (Fig. 3), previously developed for the study of VUV effects on polymers:[13,15,29,30] it consists of a hydrogen plasma "lamp," isolated from the treatment chamber by a VUV-transparent window of magnesium fluoride (MgF_2: cutoff wavelength $\lambda_c = 112$ nm). Hydrogen plasma was selected because of its very strong emission in the VUV region, comprising the Lyman and Werner series of molecular continua below about

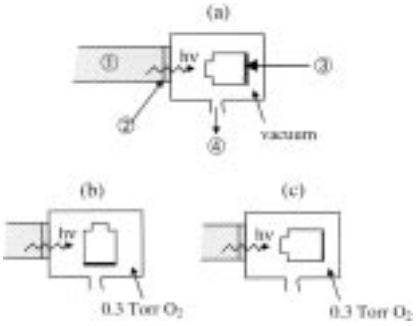

Fig. 3. Schematic representation of the hydrogen MW plasma "lamp", with a VUV-transparent MgF_2 window (p = 1 Torr, F = 100 sccm, P = 250 Watts). Vial positions for (a) VUV alone: spores in vacuum, exposed to the photon flux; (b) AO alone: spores perpendicular to the photon flux, exposed to active oxygen (AO) created by VUV in O_2, (c) VUV + AO: spores exposed to active oxygen (AO) and to the photon flux: ① H_2 plasma; ② MgF_2 window; ③ monolayer of spores; ④ to turbomolecular pump.

160 nm, and the Lyman α line at 121.5 nm. At 250 W of MW power applied to the plasma, the irradiance between 115 and 170 nm at the sample position was approximately 0.12 mW cm^{-2}.[20,30]

Inoculated glass vials were placed into the sample chamber and the methodology described by Fozza *et al.*[13,15] was used to study the effects of (a) VUV alone, (b) active oxygen species (AO) created by partial VUV absorption in O_2; and (c) the combination of both VUV and AO (VUV+AO) on spore mortality: as shown schematically in Fig. 3, exposures were carried out with the vials in vacuum, or immersed in low-pressure (300 millitorr or 40 Pa) oxygen, directly exposed to the VUV/UV photon flux (vial and irradiation axis parallel, cases (a) and (c)), or only to the VUV-generated AO (vial and irradiation axis perpendicular to each other, case (b)). All other experimental parameters were kept constant (pure hydrogen plasma, p = 1 torr, F = 100 sccm, P = 250 W). Exposure times were fixed at 5, 15 and 30 minutes. Three vials were subjected to each type of treatment, after which spores were regenerated and counted using the same procedure as described earlier. Student *t*-tests were carried out to determine statistical significance of the results at a confidence level of 95% ($p < 0.05$).

3. RESULTS

3.1. Plasma Experiments

Figure 4 presents the average spore mortality following exposure to the MW and RF O_2/CF_4 plasmas under the nominally identical conditions described before, and compared with pure O_2 MW plasma. As in the case

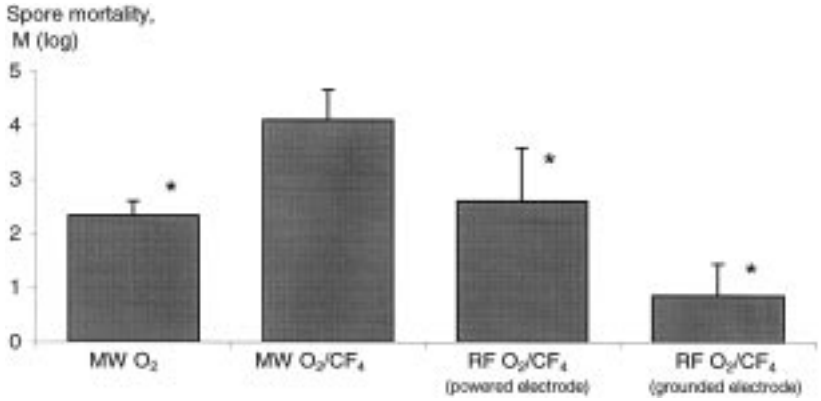

Fig. 4. Comparison of spore mortality, M, in MW and RF O_2/CF_4 plasmas, after 5 minutes of exposure (P = 200 W, p = 80 mTorr; F = 80 sccm; [CF_4] = 12%). (* significantly lower than the O_2/CF_4 MW plasma, at a confidence level of 95%; $p < 0.05$)

of polymer etching,[10,11] the efficacy of RF plasma was found to be significantly lower ($p < 0.05$) than that of its MW counterpart: M is seen to be 1.5 log lower for RF than for MW after a 5 minute plasma exposure. As expected, when the vials were placed on the grounded electrode ($V_b = 0$), the efficacy of RF-plasma dropped even further, to less than 1 log (compared with $M = 2.3$ on the powered electrode, where $V_b = -150$ V). These experiments again also confirm the significantly higher sporicidal effect of O_2/CF_4 plasma compared with its pure O_2 counterpart ($p < 0.05$).[7]

To put these results in the proper perspective, the following comments should be added : As already mentioned, the nominal power values fed to the MW and RF discharges were equal (200 W) and the visible glow zones were of similar volumes, signifying that the nominal power densities were also comparable (a few tens of mW cm^{-3}). However, it is well documented in the literature that a sizeable fraction of power delivered by an RF source to a plasma reactor may be dissipated in the matching network and transmission line. We have not measured the true RF power value delivered to the plasma in this current work, even though it has a bearing on the results presented in Fig. 4. However, other research in these laboratories, in which the true power delivered was carefully measured,[19,20] clearly showed systematic differences between MW and RF plasmas of identical volumes. On the basis of these, we are convinced that the results of Fig. 4 are trustworthy.

3.2. VUV Irradiation

The efficacy of VUV radiation and/or AO after 5 minutes of exposure is illustrated in Fig. 5. In all cases, spore mortality was very limited ($M <$

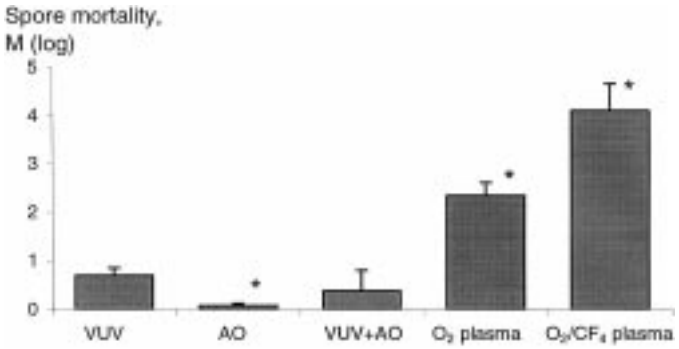

Fig. 5. Comparison of spore mortality, M, after 5 minutes of exposure to (1) VUV alone; (2) AO; and (3) VUV + AO (VUV irradiation from pure hydrogen plasma, $p = 1$ Torr, $F = 100$ sccm, $P = 250$ Watts; treatment chamber in vacuum or 0.3 Torr O_2), compared with O_2 and O_2/CF_4 plasma. (* significantly different from VUV treatment; $p < 0.05$))

1 log), much lower than after direct exposure to MW plasma (pure O_2 and O_2/CF_4 plasmas). The effects of VUV alone (samples in vacuum) and VUV + AO (samples in 300 mTorr oxygen) were found to be similar ($M \sim 0.5$ log), while AO alone (samples perpendicular to VUV radiation) exhibited even lower mortality ($M \sim 0.1$ log) ($p < 0.05$). Figure 6 shows M values after VUV and VUV + AO exposure as a function of treatment time ; even after 30 minutes of exposure, spore mortality was quite limited ($M \leq 1.6$ log). Fozza *et al.*[13,15] found that the etch rates of polymers exposed to AO alone were lower than those of VUV which, in turn, were lower than those after VUV + AO treatments. In the present study, we noted similar trends : significantly less mortality was observed with AO than with VUV and VUV + AO ($p < 0.05$). After 15 minutes or more, VUV + AO also appeared to be somewhat more effective than VUV alone, although the difference was

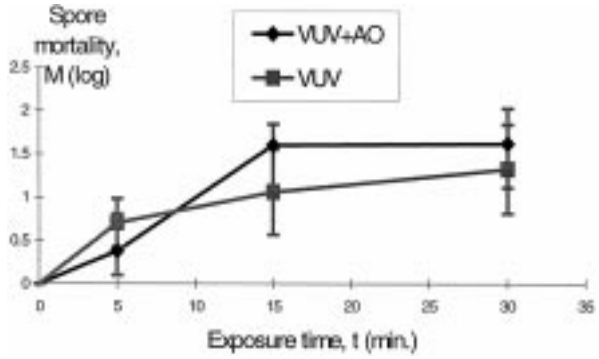

Fig. 6. Spore mortality, M, of (1) VUV radiation alone (VUV), and (2) VUV with active oxygen species (VUV + AO), as a function of exposure duration.

not significant. In all cases, *M* values remain significantly below those corresponding to direct spore immersions in the plasmas.

4. DISCUSSION

4.1. Efficacy of Plasma vs. VUV

As expected, the efficacy of MW plasma to kill *Bacillus subtilis* spores was found to be higher than that of its RF counterpart, on account of the higher concentration of reactive species (ions, radicals, . . .) in the former plasma.[19,20] It is noteworthy that even the VUV emission intensity has recently been found to be greater in MW H_2 plasma than in its RF counterpart, under otherwise identical conditions.[20] Regarding the experiments carried out in RF plasmas, greater sporicidal activity was observed when the specimens were mounted on the powered rather than the grounded electrode, presumably on account of the bias-induced ion bombardment in the former case. All these observations parallel those of etch experiments carried out with organic polymers,[11] and they therefore support our view that spore mortality in plasmas is strongly related to etching, that is, to volatilization of spore material by its chemical reaction with active species from the plasma. For further details, for example regarding the observed differences between O_2 and O_2/CF_4 plasmas and for scanning electron micrographic images of etched spores, the reader is referred to our earlier articles on this subject.[6,7]

4.2. The Effect of UV and VUV on Microorganisms

To understand the effect of plasma VUV radiation on microorganisms, we shall first refer to the considerable amount of published work on sterilization by UV irradiation, including some on radiation in the VUV spectral region. UV radiation can induce photochemical lesions in the DNA of bacteria and spores, which can lead to cell death or to mutation.[31] In the case of spores, the most resistant microorganisms to UV,[32] UV irradiation at atmospheric pressure results in a photoproduct of DNA, the thymine dimer 5-thyminyl-5,6-dihydrothymine (TDHT), commonly referred to as spore photoproduct (SP). SP damage can be corrected during spore germination, via two repair-mechanisms of DNA (≪ nucleotide excision repair ≫ and an SP-specific enzyme called SP lyase), which are considered to be important components of spore resistance towards UV, and towards other treatments.[33,34] The high observed UV resistance of spores can also be explained in part by the binding of spore DNA with small acid-soluble proteins (SASP), and by photoprotection by dipicolinic acid in the core.[35–37] Finally, spore DNA is protected by multiple layers which surround the core, namely a germ cell wall, cortex, inner and outer spore coats, and sometimes an exospor-

ium.[38–40] As shown in Fig. 7 for the case of B subtilis spores, these form a 150 to 200 nm—thick proteinous barrier, which can shield the core from the effects of UV photons. It is possible that UV radiation can also kill spores by modifying these outer layers of the spores, but the importance of this second pathway is not yet known.

It is interesting and useful to examine the reported dependencies of UV/VUV efficacy on wavelength (that is, photon energy). Particularly noteworthy in this regard is the work by Munakata *et al.*[41] who exposed various strains of B subtilis spores under vacuum to synchrotron radiation of 13 different wavelengths between 50 and 300 nm. Figure 8b shows their reported inactivation spectra, in which two main regions of high efficacy are noted, one in the VUV (around 170 nm), the other in the mid-UV (220 to 270 nm). On the other hand, radiation near 100 and 190 nm was found to be relatively ineffective.[41] If we compare this with the emission spectrum of our H_2 plasma "lamp" (Fig. 8a), the photon flux of which is comparable to that of Munakata *et al.*[41] ($\sim 10^{14}$ cm^{-2} s^{-1}), we find that the peak intensity of our VUV source ($\lambda = 160$ nm) falls into a region of moderately high inactivation (compare Figs. 8a and 8b); however, our source has a rather broad spectrum with an appreciable radiative component in the lower wavelengths (low inactivation) range, while that of Munakata is concentrated in a 5 nm-wide band around 170 nm. This has an important influence on the possible photochemical pathways and their relative contributions to the effect of the radiation,[15] and it can bring at least some partial explanation for the relatively disappointing *M* values we have presented in Section 3.2.

In this same context, German workers (unpublished data[42]) reported mortalities of various microorganisms, including Bs spores, after mid-UV irradiation with a powerful (15 mW cm^{-2}) 254 nm low-pressure mercury lamp; even though the dose was lower in the German experiments compared with ours, ($\sim$25 and 100 mJ cm^{-2}, respectively), the 254 nm radiation appeared to be much more effective, namely 1.5 log in 15 minutes, and 2.5 log in 1.5 s in this and the German work, respectively, but the latter power density was two orders of magnitude higher. Moreau *et al.*[17] showed that the

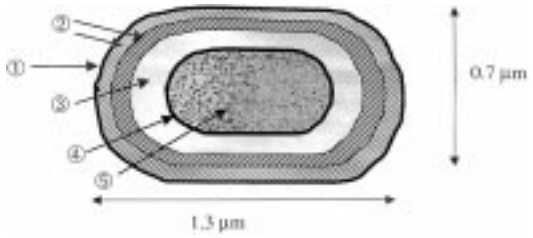

Fig. 7. Schematic representation of the morphology of Bacillus subtilis spores (after Refs. 38 and 39): ① exosporium; ② inner and outer spore coats; ③ cortex; ④ germ cell membrane; ⑤ core.

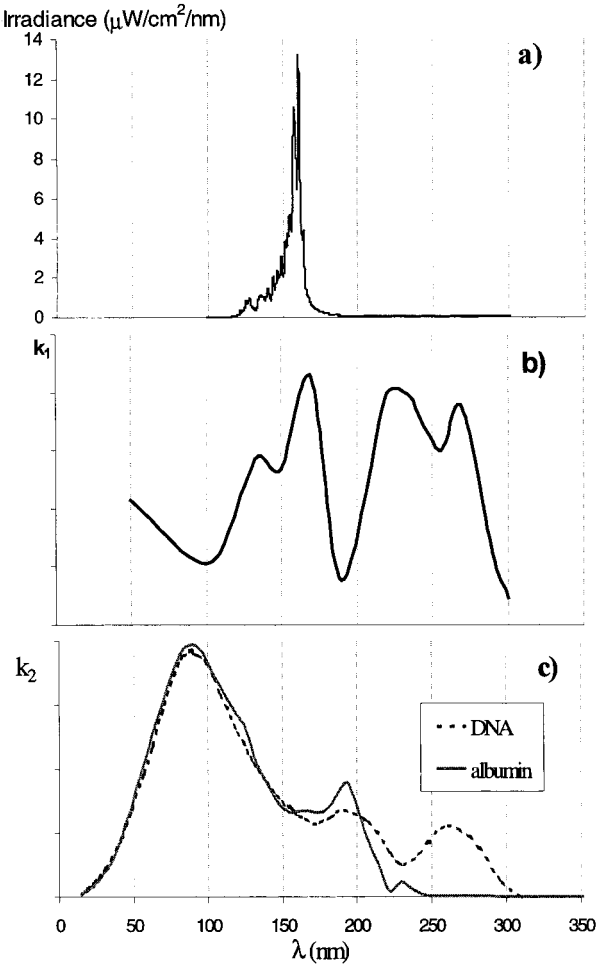

Fig. 8. Wavelength dependence of (a) VUV/UV radiation intensity from H_2 plasma, incident upon samples (after Fozza *et al.*[30]); (b) inactivation rate constant k_1 for VUV/UV radiation on spores (after Munakata *et al.*[41]); (c) optical extinction coefficient k_2 of DNA (after Inagaki *et al.*[43]) and albumin protein (after Inagaki *et al.*[44]).

UV emission of a N_2/O_2 post-discharge plasma (which has intense emission in the $300 < \lambda < 400$ nm region) plays a significant role in killing microorganisms. However, they reported much lower M values than ours. Finally, it is noteworthy that Ukrainian workers[4,28] have recently reported very high mortality of Bs spores in direct current (dc) air plasmas, namely a 6 log decrease in less than 2.5 minutes. This surprisingly large efficacy, which exceeds even our "best" results in O_2/CF_4 plasmas, is the highest ever reported.

The authors used an unusual set-up, and attributed the efficacy of their system largely to ultraviolet radiation from the dc glow discharge. They claim to have found higher *M* values with the UV/VUV emission of an air plasma (highest intensity in the 160–220 nm wavelength range, at a power density of 0.1 mW/cm^2), than with UV photons emitted from a mercury lamp (at $\lambda = 254$ nm) at a much higher power density (1.5 mW/cm^2). Yet, according to the other literature data we have cited earlier, the 160–220 nm range is not more efficient than the 254 nm radiation.[41] These surprising results certainly call for confirmation and further investigation.

Having presented the clear relationship between spore mortality and VUV/UV wavelength, one can now examine probable mechanisms underlying this relationship. In all microorganisms, DNA lesion is the main reason for ultraviolet-induced mortality; inactivation spectra are therefore correlated with the wavelength-dependent absorption coefficient of DNA, which is shown in Fig. 8c, after the results of Inagaki *et al.*[43] In the particular wavelength range that we have used here (115–160 nm), the absorption coefficient of DNA is high; yet, we found only limited *M* values. This can be explained by the fact that the inactivation spectra of spores also depend on the absorption of other spore constituents (proteinous outer layers, dipicolinic acid (DPA) present in the core etc. . .), which can absorb VUV photons and prevent them from reaching the DNA. Once again, turning to synthetic polymers, the wavelength-dependent absorption coefficients, $\alpha(\lambda)$, of a polymer allow one to calculate the penetration depth of the radiation, *d*, for particular values of λ; in the VUV range, *d* is small, typically a few tens of nm,[14] but for $\lambda > 200$ nm it can exceed 1 μm. Referring to the spore structure (Fig. 7) and to the absorption spectrum of albumin protein (see Fig. 8c), a reasonable model for the $\sim$200 nm thick proteinous outer shell layer of the spore, the VUV photons may be completely absorbed in this shielding layer. This view tends to be supported by the fact that the absorption maxima of albumin (Fig. 8c) overlap with inactivation minima (Fig. 8b) near $\lambda =$ 100 nm and 190 nm,[44] as also pointed out by Munakata *et al.*[41]

Therefore, to reach the DNA with minimal attenuation, VUV radiation must first etch the protective outer layers. Complete VUV absorption and removal of this 200 nm thick outer layer during roughly 30 minutes would require an etch rate of about 1.1 Å/s. Now, etch rates of polymers in the same apparatus and under identical experimental conditions have been shown to be much lower: Fozza *et al.*[15] have clearly correlated VUV $-$ (and VUV $+$ AO) induced etch rates, *R*, of various polymers with their $\alpha(\lambda)$ values; those with a high relative value of α in the spectral region of the H_2 plasma lamp (Fig. 8a) displayed high *R* values, while low α led to low *R* values. Polymethylmethacrylate (PMMA), the polymer with the highest *R* values among those investigated, was found to exhibit $R = 0.2$ and 0.24 Å/s, for

VUV and VUV+AO, respectively.[15] Supposing that *R* values are similar for PMMA and for the proteinous layer of spores, the exposure duration required to etch away this 2000 Å thick layer under the present VUV/AO conditions would be $2000/0.24 = 8.4 \times 10^3$ seconds, or 2.5 hours. Evidently, the use of far more intense excimer lamps[45] could greatly reduce this time, by more than two orders of magnitude. In contrast, increasing the wavelength would decrease the etch rate; thus, Esrom and Kogelschatz[45] found the *R* values of PMMA at 1 mbar due to irradiation by 10 mW cm^{-2} excimer lamp sources with 172, 222, and 308 nm wavelengths to be in a relationship of 0.035 : 0.02 : 0.01 μm min^{-1} (*i.e.*, 1 :0.57 : 0.28).

On the basis of what has just been stated, the etch rate, *R*, induced by VUV irradiation from our H_2 plasma "lamp" is too low for complete etching of spores in a reasonable time. This contrasts sharply with what we observed in the MW O_2/CF_4 plasma,[6,7] where *R* of polymers such as PMMA or polyimide (PI) have been shown to be typically 0.5 μm min^{-1} (~80 Å/s) under the conditions used,[10] that is, 400 times higher. However, this does not allow us to conclude from our study whether DNA damage or damage to spore membranes is responsible for the significant mortality (>90%) induced in the present VUV irradiation experiments. While DNA damage appears to be clearly demonstrated as the mechanism of spore destruction in the highly efficient mid-UV (or UV-C, e.g., at 254 nm), the mechanism for vacuum-ultraviolet irradiation is thought to be different[41,46] and still subject to controversy. Two possible explanations may be the following: (1) even though the radiation is very strongly (exponentially) attenuated in the outer spore layer, some VUV photons can nevertheless penetrate into the core and alter the DNA, as also concluded by Munakata *et al.*[41]; (2) even though *R* is too low to completely etch spores, membrane damage induced may be sufficient to kill them. Thus, in yeast cells, microorganisms with a much larger diameter but thinner membranes than spores, the major cause for lethal damage was found to occur in the membrane, not in the DNA.[46,47] The absence of any match between the inactivation spectrum and the absorption of DNA in this VUV region (compare Figs. 8b and 8c) does not permit validation of this second hypothesis until further data are in hand. It is conceivable that the two processes leading to spore mortality by VUV photons may even occur simultaneously. In the case of direct exposure to either O_2 or O_2/CF_4 plasma, VUV emission (e.g., 130 nm radiation from atomic oxygen) will certainly also contribute to the observed spore mortality, especially once the outer membrane has been significantly thinned by the etch process.

In the region of lower photon energies ($\lambda > 200$ nm) DNA absorbs fairly weakly (Fig. 8c), as we have already noted, but so do the proteinous outer layers ; this may explain the high efficacy in the UV-C wavelength

range, for example the previously mentioned experiments at 254 nm. Indeed, this resonant radiation from low-pressure mercury lamps has been used for surface desinfection for many years.[22] This UV regime above 200 nm, where photon penetration depths are at least comparable to spore dimensions (see Fig. 7), has the important added convenience and cost-saving characteristic that it does not require irradiation to take place under vacuum or protective gas environment. However, all UV lamps have drawbacks for the treatment of complex geometries, in that the radiation travels on a line-of-sight pathway, and is therefore limited by shading effects. Chemically active species from plasma, on the other hand, are not subject to this limitation; they can penetrate into small cavities and obstructed regions of the exposed medium, which may help explain their greater effectiveness that we have reported here.

5. CONCLUSIONS

In this research, we have shown that MW plasmas are more effective than their RF counterparts in killing Bs spores, under otherwise nominally identical conditions. Although the real power deposited in the RF discharge may be lower than in its MW counterpart, we feel that this result is trustworthy, that it can be related to the higher etch rate in the former plasma, and that it confirms the major role of etching in the destruction of spores directly exposed to the plasma.[6,7] In contrast, the etch mechanism plays a much less clear role in the case of VUV/UV irradiation.

Biological effectiveness of UV/VUV radiation varies markedly with wavelength and possibly with power density. VUV/UV emission from plasma, in turn, depends on experimental parameters such as gas composition, pressure and power.[29,30] This can partly help to explain the current controversy concerning the role of UV in plasma sterilization. In this particular work, we have studied the efficacy of intense VUV emission from H_2 plasma in the range from 115 to 170 nm, so that our conclusions must be limited to this particular wavelength region.

Our hypothesis has been that the very energetic VUV photons emitted in a glow discharge plasma may have a greater effect on spores by attacking not only DNA but also the spore membranes. However, we have found the Bs spore mortality induced by 0.12 mW cm^{-2} of VUV radiation from 115 to 170 nm to be rather limited: more than 5 minutes of exposure was necessary to kill 90% of the microorganisms, and more than 30 minutes to kill 99%; only marginal improvement was obtained with the simultaneous presence of AO. VUV irradiation therefore does not appear very promising, since it is not more efficient in killing microorganisms than UV-C,[41] while being more susceptible to damage the surface layers of polymer-based medical

devices.[12–15] In contrast, low-pressure plasmas with high etch rates and intense emissions in the UV-C region would be advantageous by offering both mechanisms for destroying microorganisms.

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