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Atmospheric Plasma Sterilization and Deodorization of Dielectric Surfaces

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A method is presented for rapid and uniform sterilization and deodorization of dielectric surfaces. The technology is applicable to the inside surface of PET or glass bottles, polymer caps, plastic tubes, etc. The treatment is based on a pulsed RF discharge in air at atmospheric pressure (eventually with addition of argon) creating a nonequilibrium plasma on the treated surface. The plasma effectively destroys microorganisms in vegetative or sporulent form. It also slightly etches the polymeric material, removing some atomic layers and, thereby, cleaning it from aromatic organic components (deodorization). The process is short: PET bottles 1.5 L, in particular, can be treated in about 20 msec. The results of surface analysis and microbiological, chromatography, and spectroscopy tests are discussed. A device has been developed and integrated into an industrial-filling machine for on-line sterilization and deodorization of the inside surface of PET bottles before filling, and for sterilization of caps and bottle necks before seaming. It allows cold asceptic filling at a rate of 36,000 bottles per hour.

KEY WORDS: Atmospheric plasma; RF; 20-msec pulses; sterilization; deodorization; PET bottles; inside surfaces.

1. INTRODUCTION

The subject of plasma sterilization has been mainly explored at a laboratory level.[1–3] A breakthrough in the industrial application of sterilization of medical instruments and materials by means of vacuum plasma has been obtained. Machines such as "Sterrad"[2] and "Plazlyte"[3] using RF vacuum plasma for this purpose are now commercially available.

Recently, a substantial effort has been made to perform surface sterilization by means of atmospheric plasma (corona, barrier discharge) technology.[4] It has been possible to realize a reduction of 10^6 microorganisms in vegetative and spore forms, in tens of seconds, on flat surfaces.

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The process of sterilization by plasma is not yet well understood. Oxidative processes in air plasma containing active oxygen atoms, molecules, and radicals, can damage the microorganism membrane, destroying its DNA and proteins.

More generally, four mechanisms are responsible for killing microorganisms: (1) plasmochemical reactions, such as oxidation and etching; (2) electron bombardment; (3) UV irradiation; and (4) surface ablation.

The efficiency of plasmochemical reactions depend on the chemical composition of the plasma and the specific energy input. Both factors determine the density flow and the degree of activation of the plasma particles reaching the microorganism surface. The characteristic energy of the plasma particles, as a rule, is much higher than the binding energy of the organic molecules of the microorganism. Therefore, these molecules are easily destroyed. For this reason, even a simple argon plasma should have a sterilization ability. Moreover, plasmochemical reactions between the plasma particles and the organic surface of the microorganism can substantially accelerate its destruction. For example, an oxygen or air plasma should destroy (etch) the microorganism surface by oxidation, forming residual volatilizing CO_2 and CO particles.

Electron bombardment is important if the microorganisms are lying on a surface, or suspended in a volume, and is crossed by the electrical current generating the plasma. The efficiency of this process, obviously, depends on the current density and the electric field in the vicinity of the surface.

UV irradiation, which accompanies any plasma decay, will complete the sterilization. Its efficiency depends on the radiation intensity and is determined by the process of plasma generation and composition.

Ablation is an hydrodynamical phenomenon accompanying any intensive dense plasma flow treatment of the surface. In this case, the microorganisms are blown away together with the material stripped from high-temperature region of the treated surface, where they are destroyed.

To perform the described processes, plasma should preferably be generated in a nonequilibrium state. Two competing methods exist: vacuum plasma and atmospheric plasma generation.

In vacuum plasma, the mean free path of the plasma particles is large compared to the device dimensions. In this case, electron bombardment can be the dominant process of sterilization. Moreover, a nonequilibrium plasma state can easily be obtained, even in a continuous large volume mode of generation. However, the plasma enthalpy and the particle flow densities are relatively low so that plasmochemical reactions and UV radiation are not intense, ablation is absent, and, as a result, the duration of a vacuum process treatment must be large for it to be efficient (typically tens of seconds, minutes, and sometimes hours).

In atmospheric plasma, the plasma is a continuous high-enthalpy medium in which nonequilibrium can only be reached either in short pulses or in the presence of large gradients of temperature, concentration, and mass-flow velocity (for instance, in boundary layers). Electron bombardment is limited due to a relatively small electron mean free path, but plasmochemical reactions and UV radiation are intense and ablation becomes significant. Consequently, the duration of such a surface treatment can be small (milliseconds).

Another substantial difference is that rarefied plasma needs vacuum devices (pump, chambers, valves, etc.), which makes the installation much more complicated, expensive, and difficult to adapt to real industrial applications than atmospheric plasma.

In the present work, an attempt has been made to apply atmospheric plasma process for sterilizing dielectric surfaces, like polymers, which are thermophobe and cannot be disinfected by standard thermal methods. A solution has been found for sterilization not only of flat surfaces but of complicated, inaccessible surfaces like the inside surface of plastic bottles or tubes. It is shown that cleaning, and, in particular, deodorization of the surface can be performed simultaneously with sterilization. Efforts have been concentrated on rapid sterilization, lasting tens of milliseconds.

2. FEASIBILITY EXPERIMENTS ON FLAT SAMPLES

Principle experiments were performed using an Ar plasma jet in air produced at atmospheric pressure by means of a multijet generator shown on Fig. 1 and described in detail[5–8] (plasma funnel).

According to Ref. [6], the plasma jet parameters at different distances from the generator nozzles were estimated as shown in Table I.

Flat substrates (diameter, 100 mm) composed of different materials (steel, glass, polymers) were passed through the plasma jet, perpendicularly to its axis, at different speeds (0.3–2 m/s). The exposition time to plasma varied between 0.01 and 0.15 s. The sample temperature during the treatment, measured by radiometry, did not exceed 65°C.

Different kinds of microorganisms (*Bacillus subtilis*, *B. stearothermophilus*, *B. pumilus*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*) were deposited on the samples in the form of spots (diameter, 3–4 mm) containing from 10^3 to 10^6 microorganisms per spot, i.e., from 3.10^4 to 3.10^7 microorganisms per cm^2. The plasma parameters were adjusted for the different substrate materials so that all the microorganisms were destroyed, even when they initially formed multilayers.

SEM photos (Fig. 2) and AFM photos (Fig. 3) illustrate the influence of the plasma treatment on the morphology of spores (*Bacillus subtilis*) still

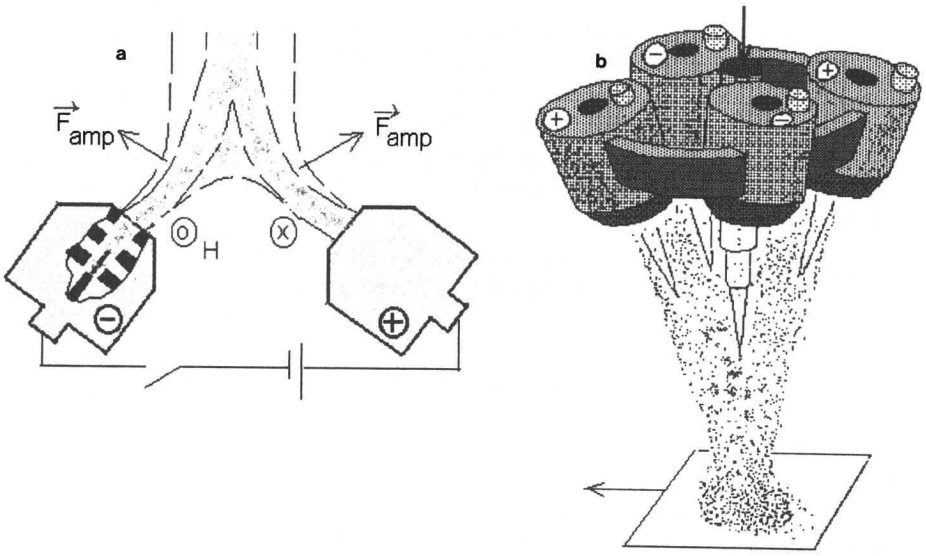

Fig. 1. Scheme of the multijet plasma generator used for sterilization experiments. (a) Twin-torch generator; (b) plasma funnel, made of two twin-torch generators.

remaining on the surface and not blown away by the plasma flow. It seems reasonable to explain the action of the plasma on the microorganisms remaining on the treated surface as a weakening of the cell membrane, because of surface etching in oxygen plasma, and leading to fatal local membrane tearing.

In the case of steel and glass, the substrate surface was not altered by the plasma sterilization treatment. On the polymeric substrates, a surface-polishing effect was observed, which is illustrated on SEM photos (Fig. 4)

Table I. Plasma for Parameters

Parameter	Distance from the generator nozzles (cm)	
	2	10
Axial temperature (K)	~11,000	~6500
Axial mass flow velocity (m/s)	~120	~35
Plasma jet visible diameter (cm)	~3	~4
Plasma composition		
Ar (%)	~50	~20
Ar (%)	~50	~80

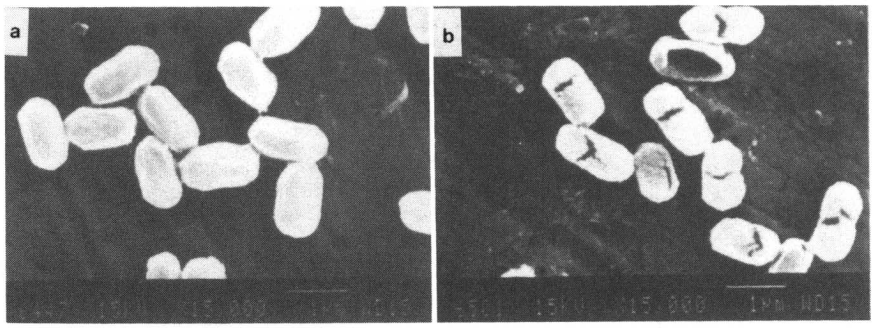

Fig. 2. SEM photos of spores (*Bacillus subtilis*) treated by an atmospheric plasma jet. (a) Before treatment; (b) after treatment.

made before and after treatment of the PET surface of a multilayer packaging material. It is seen that pores, cracks, and surface relief are eliminated by the treatment. The scale of the polished relief appears to be $\leq 10\,\mu m$. It is assumed that such a polishing effect is due to an ablation phenomenon.

ESCA measurements show that the treated surface is partially oxidized and lightly nitridized within a layer, not exceeding 100 Å. This indcates that plasma etching is also a probable process modifying the polymeric surface. This etching property was used for surface cleaning and, in particular, deodorization.

A special aromatic product, limonen, known for its ability to penetrate the polymer, to some extent, and to transmit, even in very low concentrations, a taste to a liquid in contact with it, was deposited on a polymer (PET) surface and treated by plasma jet. The parameters of the plasma flow were chosen so as to minimize ablation. After treatment, the limonen concentration, measured by chromatography, was reduced by a factor of 10 (see Fig. 5). This confirms the feasibility of surface deodorization and cleaning using this type of treatment.

3. RF FLASH PLASMA

3.1. Flash Discharge

For many industrial applications, such as the treatment of cavities (bottles, long tubes, etc.), jet plasmas are not the optimal solution. Often, rapid treatment is required. For example, sterilization of PET bottles should be operated at a rate of 10 bottles per second. Because of large hydrodynamical resistance and energy losses, a suitable plasma jet cannot

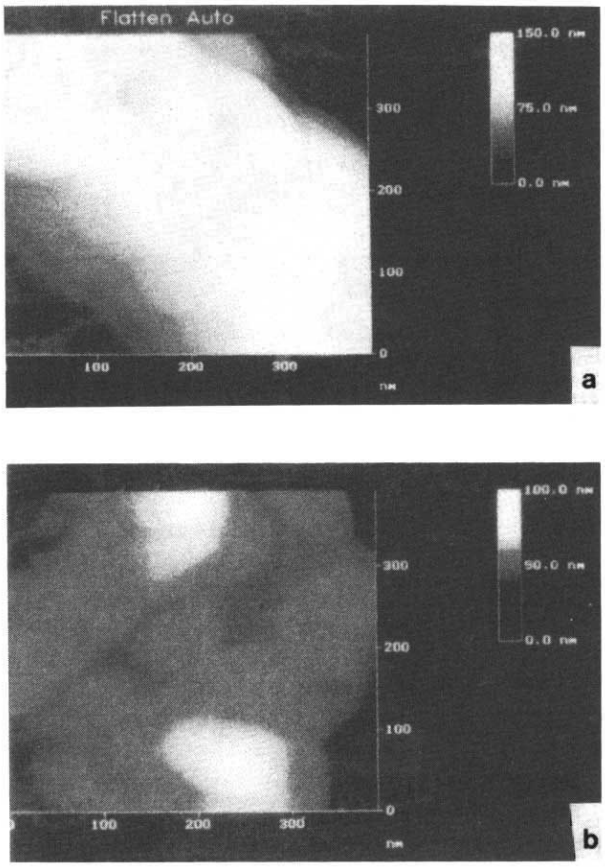

Fig. 3. AFM pictures of spores (*Bacillus subtilis*) treated by an atmospheric plasma jet. (a) Before treatment; (b) after treatment.

penetrate a bottle. Introducing a plasma duct, an electrode, or a mini-plasmatron into a bottle or a tube is technically complicated, slow, and expensive. Atmospheric pressure corona or barrier discharges, which are low-current (typically tens of milliamperes[4]) high-voltage discharges are too slow, also.

To overcome the problem, a radio frequency (RF) high-current discharge is used in such a manner that no electrode is introduced into the bottle and the bottle walls themselves stabilize the plasma.[9] In other words, the bottle is used as a plasma generator, and the plasma generated in it, instead of being introduced into the bottle is, in fact, ejected from the bottle. Figure 6a illustrates the general principle.

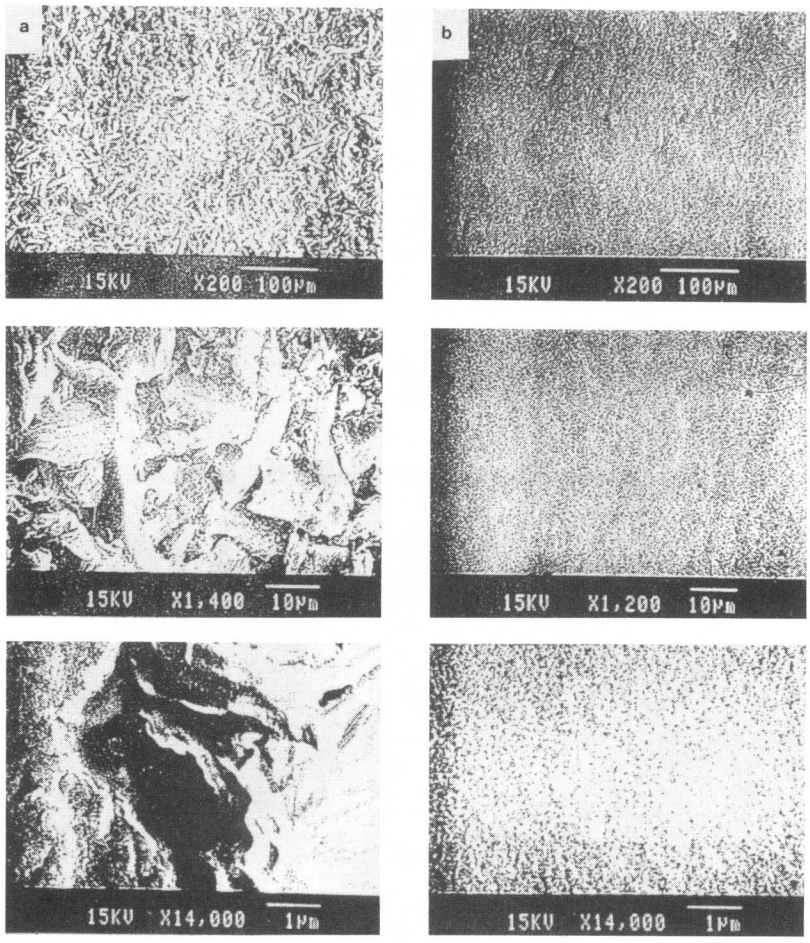

Fig. 4. SEM photo of the PET surface of a multilayer packaging material, treated by an atmospheric plasma jet. (a) Before treatment; (b) after treatment.

A 1.5 L bottle, for example, was treated using the following parameters: mean input power, 20 kW; ignition voltage, 15 kV; mean pulse current, 10 A; pulse duration, up to 20 ms; and RF frequency, 3 MHz.

The discharge is initiated by means of a central electrode, located outside of the bottle above its neck. A second, external, grounded electrode surrounds the bottle. A coaxial insulator is designed so as to ensure a uniform distribution of the current over the entire inside bottle surface, so that the current lines should cross the treated surface under an angle close to 90°, even in the case of a complicated bottle geometry (see Fig. 6b). The

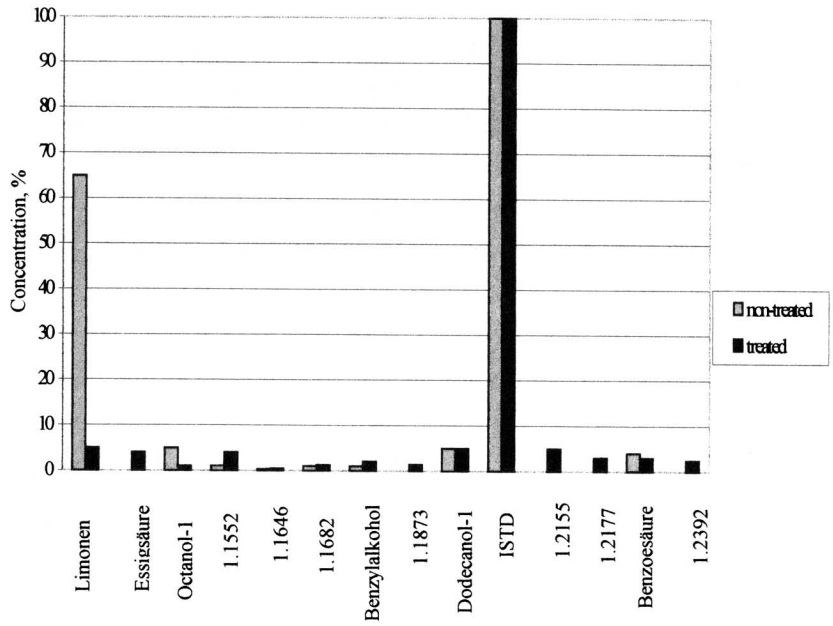

Fig. 5. Chromatogram illustrating the reduction by a factor of 10 of the surface concentration of limonen on a PET surface treated by an atmospheric plasma jet.

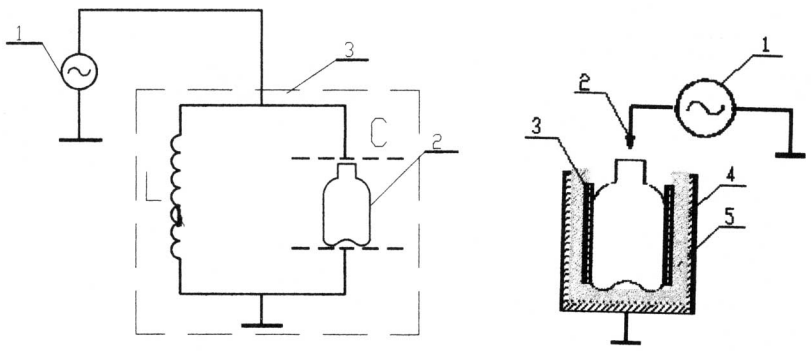

Fig. 6. Scheme of flash RF plasma generation in a dielectric bottle. (a) General principle: (1) RF pulse generator; (2) bottle; (3) RLC circuit load. (b) Plasma device: (1) RF generator; (2) central ignition electrode; (3) stainless-steel screen; (4) grounded electrode; (5) dielectric wrapper.

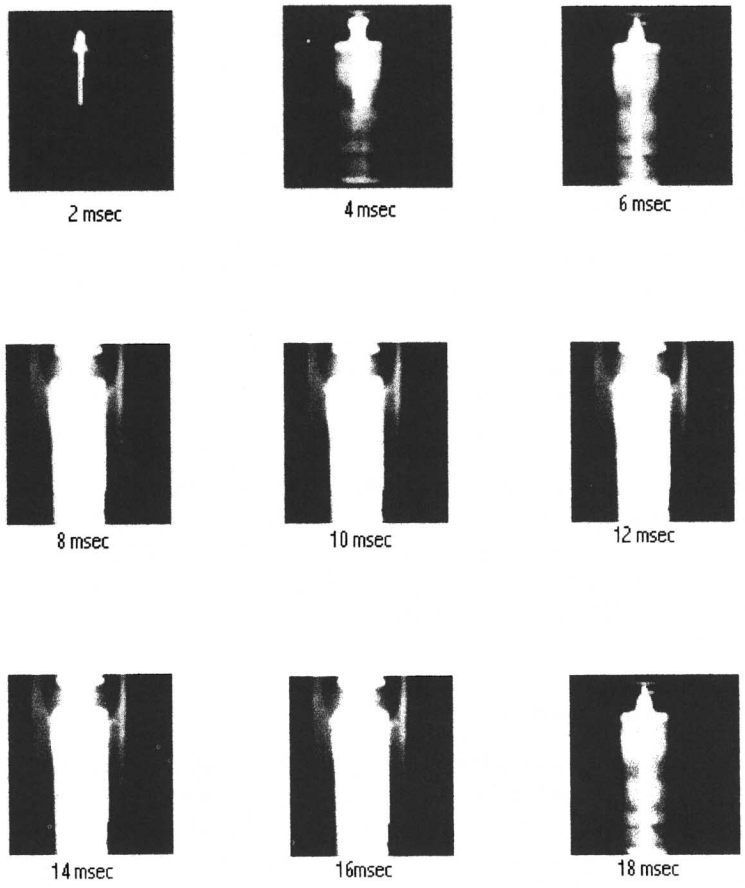

Fig. 7. High-speed frames of the plasma generation in a 1.5 L PET bottle. The frame frequency is 500 kHz.

plasma is generated in the air contained in the bottle before treatment. A small portion of argon is eventually injected through the central electrode to facilitate and localize the plasma ignition.

Figure 7 shows high-speed frames of the light emission of a plasma generated in a PET (1.5 L) bottle. It can be seen that the plasma is generated first at the axis of the bottle, then, during 4–6 ms, propagates to the wall, uniformly fills the bottle for about 10 ms ejecting a jet from the bottle, and then vanishes in the inverse sequence. During the process, the bottle wall temperature, measured by a low-inertia thermocouple, immediately after the RF flash, does not exceed 50°C.

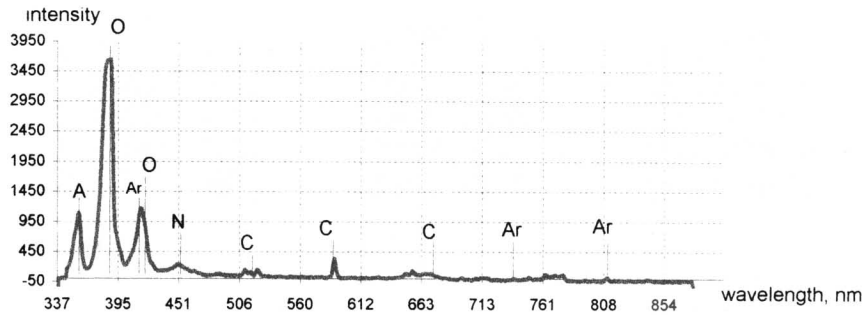

Fig. 8. Optical emission spectrum of the flash plasma, showing, in addition to the lines of argon and atomic oxygen and nitrogen, the presence of carbon atoms resulting from PET material etching by the oxygen of the plasma.

Similar experiments have been done on glass and PET bottles at different volumes (up to 1.5 L) and on plastic tubes with a diameter varying between 0.3 and 10 mm and a length between 0.5 and 10 m. The results of the tests, for the purpose of industrial application of sterilization and deodorization of bottles (in particular, PET bottles) are reported further.

3.2. Light Emission Spectrum

The light emission spectrum of the plasma jet ejected from the treated bottle has been registered by means of a spectrometer (SD2000 Ocean Optics) in the wavelength range 330–860 nm (Fig. 8). The characteristic lines of Ar_I, O_I, and N_I atoms are present. An important part of the plasma radiation is emitted in the UV range.

A small amount of carbon is identified proving that the PE material is slightly etched by the oxygen components of the plasma. No emission of metals atoms (central ignition electrode: Cu, Ag) is detected.

3.3. Microbiology Tests

Preliminary tests were made using the following microorganisms: spores (*Bacillus subtilis*), yeasts (*Saccharomyces cerevisiae*), molds (*Aspergillus niger*). The tests showed that a concentration of 10^4 microorganisms per bottle are fully destroyed by the plasma treatment.

The main tests were made with *Byssochlamys nivea*. This microorganism is characteristic for beverages. It is stable in acid liquids ($pH < 5$) and at temperatures up to 98°C.

The following two procedures were used:

1. Bottles (110 PET) were filled with distilled water containing 10^6 microorganisms per liter and then dried. Microorganisms (10^3–10^4) were counted on the inside bottle wall. Ten bottles were used as references (untreated). The remaining 100 bottles were treated by plasma. The subsequent microbiological count showed that all the treated bottles were fully disinfected (see Fig. 9), indicating a reduction rate of more than 10^3 per bottle.[4]
2. Spots (~1 cm^2) containing ~10^6 microorganisms were deposited at different places on the inside wall of the bottle (neck, body, bottom). After treatment, counting showed full microorganism destruction.

After treatment, it is difficult to find microorganisms on the treated surface closest to the bottle neck, which indicates that an important convective flow is involved. Some killed microorganisms can be found mainly on the bottom of the bottle.

Following treatment, the volume of microorganisms is substantially increased: their surface is damaged and holes are observed, through which the cytoplasm has been ejected during the microorganism destruction (Fig. 10c and d). It is obvious that, before rupture, the membrane was first weakened, probably due to plasma etching.

3.4. Surface Chemical Composition Tests

To test the deodorization ability of the plasma flash[5], PET bottles were filled with water, containing 200 mg of limonen per liter and held in a thermostatic chamber at 50°C for 84 hr. Afterward, the bottles are emptied, dried, and treated by flash plasma. Samples of 100 cm^3 were taken from different parts of the bottle and submitted to gas chromatography. Desorption in a nitrogen atmosphere at 140°C was performed. Measurements showed that the limonen concentration on treated bottles was reduced by a factor of more than 100, compared with untreated bottles (see Fig. 11).

Attention was also focused on the eventual formation of harmful products on the PET surface, due to the plasma treatment. Various analyses[6] were performed to check for a change in PET material itself and the organic compounds contained in the PET material after plasma treatment. Three

[4]The tests were made at the "Service de Lutte contre les Nuisances," Section des Toxiques et Substances, dangereuses pour l'Environnement, Les Croisettes, 1066 Epalinges, Switzerland.
[5]The chromatography tests were made at a specialized laboratory at the "Ecole Suisse d'Ingénieurs des Industries Graphique et d'Emballage," Lausanne, Switzerland.
[6]Analysis at BON Technologies SA, Saint-Prex, Switzerland.

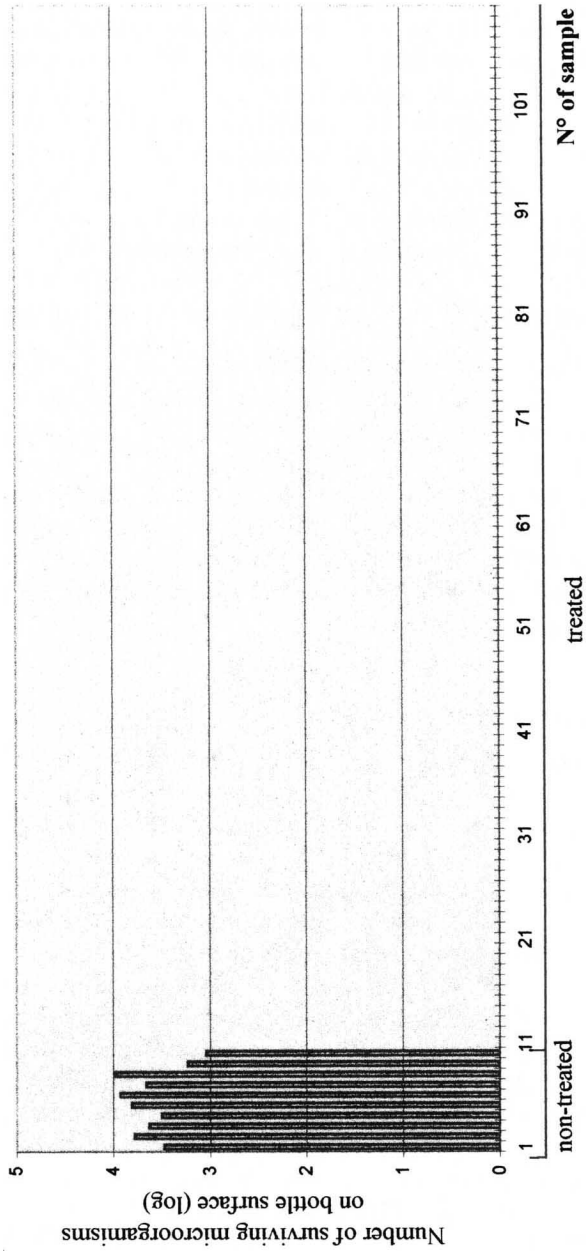

Fig. 9. Results of microbiological tests. Number of surviving microorganisms (*Byssochlamys nivea*) on untreated (1–10) and consecutively treated 1.5 L PET bottles (11–110).

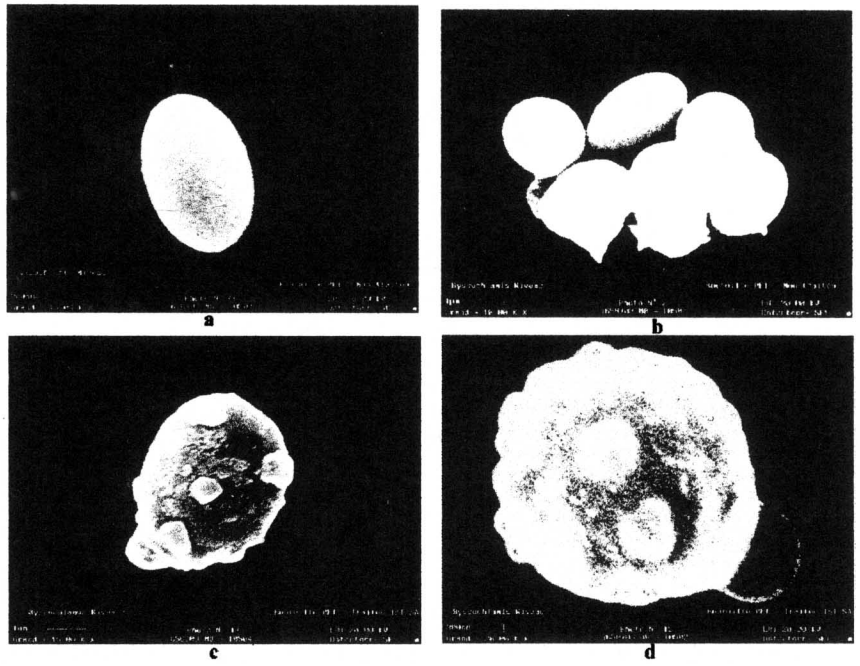

Fig. 10. SEM photos of *Byssochlamys nivea* before (a, b) and after (c, d) plasma treatment.

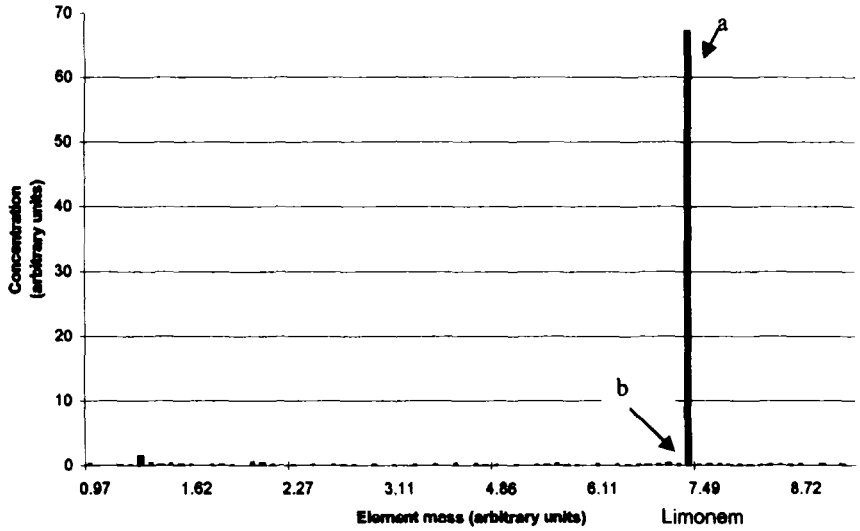

Fig. 11. Results of chromatography measurements of the surface composition of PET bottles contaminated by limonen. (a) Before treatment; (b) after treatment.

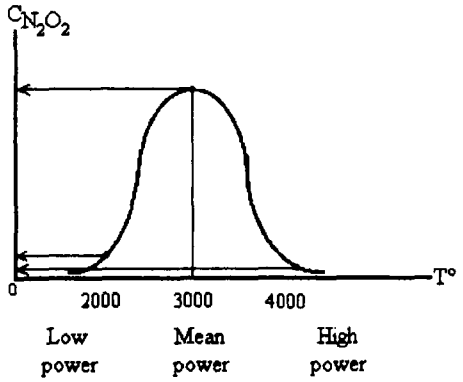

Fig. 12. Equilibrium N_2O_2 concentration dependency on temperature. The arrows illustrate the possible processes of N_2O_2 composition freezing. Low values of N_2O_2 concentration can be obtained by freezing low-power and high-power plasma.

methods were used:

1. GI permeation chromatography, which gives the molecular weight distribution of PET material.
2. Photoelasticity tests, made by means of monochromatic ($l = 589$ nm) polarized light (indicating stress in PET material).
3. Gas chromatography was used to quantitate organic compounds in water stored in PET bottle using the following tests: (a) Detection of acetaldehyde diluted in water. This compound is typically a very volatile product, which is formed in PET material bulk during the high temperature bottle blowing. It is responsible for a characteristic plastic odor in the stored liquid detectable by nose from a level of 20 ppb. (b) Identification and quantification of semivolatile organic compounds.

The results of the comparison of treated and untreated PET bottles are as follows: no degradation of the polymer itself; no changes in the stress of PET material; no detectable changes of organic components diluted in water stored for 1 month.

3.5. Residual Gas Compounds

Normally, an atmospheric plasma discharge in air is accompanied by the formation of ozone (O_3), nitrogen oxide (N_2O_2) and NH_3 (the last is probable if the discharge is operated in wet air) (Fig. 12). Using a powerful RF pulse and rapid ($< 10^{-2}$ s) plasma cooling (quenching), it is possible

Table II. Impact of Residual Gas Compounds and Plasma Sterilization

Compounds	Reference (untreated bottle) (71 mg)	Treated bottle (mg/L)	Legal limits (mg/L)
O_3	0	0	0.05
NO_3	< 0.1	< 0.1	40
NO_2	0	< 0.1	0.1
NH_3	0.01	0.01	0.5

to avoid or to substantially reduce the generation of these harmful gases. Nevertheless, in practice, a small quantity of ozone and nitrogen dioxide, and eventually NH_3, are produced during the RF flash discharge in a PET bottle, which causes a characteristic odor after treatment.

In order to check the eventual impact that such gases may have on the beverages, the bottle was filled just after treatment with distilled water, then closed, shaken, stored for several hours, and then submitted to chemical analysis[7]. The results are shown in Table II. They show that there is no detectable ozone and ammoniac and that the concentration of nitrogen oxide ions is much lower than the allowed limit.

4. INDUSTRIALIZATION

A device has been built and integrated into an industrial-filling machine for on-line sterilization and deodorization of the inside of PET bottles before filling and for caps and bottle neck sterilization before seaming.[10] The machine is designed for a treatment and filling rate of 36,000 bottles/hr. The method allows cold, aseptic filling. The estimated cost (investment and operating cost) of this system is substantially lower than systems based on other methods.

5. CONCLUSIONS

Atmospheric plasma has been demonstrated to be effective for uniform surface sterilization in extremely short times (tens of milliseconds); surface etching of polymeric material; PET bottle deodorization; treatment of complicated surfaces (e.g., bottles and tubes).

The process has no harmful effect on treated bottles or on beverages filled into the bottles after treatment.

[7]All the microbiological manipulations and the counting were made by an independent laboratory "Ecobion SA" (Carouge, Switzerland) accredited for microbiological analysis of food and other products according to the ISO9002 standard.

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