STUDY OF OXYGEN PLASMA FOR APPLICATION IN STERILIZATION PROCESSES

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Abstract: The main objective of this work was to propose a technique of sterilization for medical devices with less exposition time than current plasma techniques, and also determine if this technique can be applied to temperature sensitive materials. Therefore, it was used as biological sensor Bacillus subtilis spores var. niger ATCC 9372 and Bacillus stearothermophilus. For Bacillus subtilis indicators were used two substrates: glass with 2,0 x 107 CFU/substrate of microbial load initial, and paper strips with 3,8 x 10^6 CFU/ substrate of microbial load initial. The efficacy of process was evaluated with the count of survivors and it respective value of decimal reduction (D value). In this work it was used RIE (Reactive Ion Etching). For all processes were used Petri dishes with the sample in triplicates for both microorganisms types. The process parameters was varied as follow: gas flow - 100, 200 and 500 sccm, pressure – 100 and 330 mTorr, RF power – 50, 100 and 150 Watts and the time were of 2 minutes up to 60 minutes. After these processes, we made the count of survivors, in order to evaluate the plasma efficiency as sterilizer agent. Espectrophotometric analysis was made to evaluate the oxygen consumption during all process, and was used a scanning electronic microscope to visualize the plasma effect over microorganisms. With these results it was possible to adapt the process parameters for each type of substrate.

1 INTRODUCTION

Sterilization processes aim at full elimination or destruction of microorganisms (viruses, bacteria, fungi in sporulated or vegetative state) in a certain material to attain an acceptable security level for both patients and operators. Sterilizing methods can be divided into physical, chemical or physical-chemical. Physical processes are e.g. saturated steam/autoclaves, dry heat/ovens, and radiation/gamma rays.

These methods' main problem is the high temperature that can degrade polymeric materials used in catheters and other devices. This limitation affects materials used in endoscopy and a wide variety of plastic and elastomeric materials used in a wide range of clinical, therapeutic and surgical techniques (Holy, 2001).

Plasma sterilization techniques provide a wide range of advantages compared with other methods used, because they can be effective in microbial load, besides working at room temperature and not using toxic gases. They expose materials with microorganisms to reactive species generated by a gas ionization (generally oxygen), using electromagnetic fields (Lerouge, 2000; Oshma, 1997; Hermelin, 1998; Rutala, 1999).

Ideal characteristics for a low temperature sterilization process include the following: high efficiency, fast action, great penetration power, compatibility with the largest number of materials possible, non use or generation of toxic products, efficiency even with organic material, easy adaptation in each environment (hospital and industry), possibility to be monitored and easy operation (Hoefel, 2002).

Considering those items, the best method approaching the ideal is the plasma process; however an effective method using plasma as the main sterilizing element has not been developed.

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Current equipment use the plasma step to eliminate toxic residues generated by hydrogen peroxide or peracetic acid sterilization methods, as this plasma step is only performed at the end of the sterilization cycle (Hayakawa, 1998). Hence, there does not exist a specific method to use plasma for an effective sterilization, neither does there exist commercial equipment that allows using this sterilization method (Moisan, 2001).

Furthermore, possible limits regarding aspects of validation are known, be it on the dimensional limits of the sterilization chamber, be it on the configuration of the product. The reasons for these concerns are the homogeneous distribution of the formed plasma and the average lifetime of its constituents, which are known to be unstable.

2 EXPERIMENTAL

Reactive Ion Etching (RIE) system consists of a stainless steel chamber with 330 mm of diameter and 114 mm high. Inside is a 6-inch diameter electrode, which creates the plasma. The electrode is cooled at 20 °C, maintaining a constant temperature during the processes. The Radio Frequency for the generation of the plasma is 13.56 MHz; at this frequency, strong ion bombardment and high electric fields are the typical plasma characteristics.

In order to compare the process results, other sterilization tests were performed in an autoclave, Lutz Ironing, Model 39.211, with 6 kg/h of vapour production, 5 KW of power, and an X-ray Diffraction equipment, model URD6, with a molybdenum tube and a power capacity of up to 1000 W.

The indicators of *B. stearothermophilus*, inoculated on paper, with an initial load of 1 million UFC/ml, were submitted to plasma exposures at the following process conditions: 500 sccm of oxygen flow, 330 mTorr pressure, 100 W power and process times of 5 up to 60 minutes. For the indicators of *B. subtilis*, inoculated on glass plates, the gas flow was 200 sccm, for a pressure of 100 mTorr, and process times of 2 up to 120 minutes. Power levels of 100 W and 150 W were applied for each flow and pressure parameter. For all the processes, oxygen was injected into the chamber for 10 minutes before igniting the plasma, to guarantee that the chamber was full filled with this gas.

The sterilization process in the autoclave was performed for 15 minutes at a temperature of 121 °C.

The sterilization processes using X rays were done during 30 minutes, with power levels of 200, 400, 600, 800 and 1000 W. Finally, the UV processes were performed during 1, 5, 10, 30, 45 and 60 minutes, with an effective lamp power density of 14 mW/cm^2; hence the effective dose, being the effective power multiplied by the exposure time, was varied between 0,84 J/cm² and 50.4 J/cm²

The indicators of *B. stearothermophilus* used in the plasma sterilization processes, were produced on cellulosic strips of 50 mm by 5 mm with loads of 1.0 x 10^6 UFC/strips, and fabricated by the Steris Corporation. The celulosic strips, which support the *B. subtilis*, have dimensions of 40 mm by 3 mm and an initial load of 3.8 x 10^6 UFC/ml, fabricated by 3M. The indicators deposited on the glass plates, with dimensions of 18 mm by 18 mm, have an initial load of 2.0 x 10^7 UFC/glass and were fabricated by the college of Pharmaceutical Sciences of the University of São Paulo.

The procedure of counting the surviving spores was made like follows: first the spores had to be removed through mechanical agitation in test tubes with sterile water; then they were stirred in a bath of water at 70 °C for *B. Suitilis* and 82 °C for *B. Stearothermophilus* during 15 minutes. These samples were then diluted and 1 ml of these dilutions were placed on Petri dishes together with a culture of agar casein soy and placed in an oven. Incubation for the *B. Stearothermophilus* indicators was done at 45 °C during 72 hours and for the *B. Subtilis* indicators at 37 °C during 24 hours.

3 RESULTS AND DISCUSSIONS

Figure 1 shows the results of the RIE sterilization processes, using *B. stearothermophilus* indicators, inoculated on paper. It's possible to observe that after only 7 minutes, there already occurred significant reduction of microbial load. The process was performed in the following way: Petri glass dishes with strips containing *B. stearothermophilus* were placed, without a cover, inside the reactor. The pressure was lowered down to 10^{-3} Torr and the process gas was injected. The process duration varied from 2 to 60 minutes, with power level of 100 W, oxygen pressure of 330mTorr and oxygen flow of 500 sccm.

Figure 2 shows the relation of *B. stearothermophilus* samples before and after suffering an RIE type plasma sterilization process and a comparison with the autoclave sterilization

process. It can be observed that the plasma process is much more aggressive than the autoclave process. Each sample was analysed by scanning electron microscopy. After two minutes of plasma processing, only a few microorganisms could still be found on the paper, and many of those had their morphology modified. After 5 minutes of processing, the concentration of modified spores was higher. After 7 minutes, a lot of spores had been destroyed and the few that remained had a completely changed morphology. After 10 minutes of sterilization, it was very difficult to find any spore; many spores were split into several fragments. These results show that the process is efficient to sterilize these biological indicators. However, it is necessary to be careful with the time of exposure to the plasma, because for times higher than 15 minutes, the cellulose material starts to suffer degradation and it disintegrates completely after 30 minutes of plasma (Moreira, 2003).

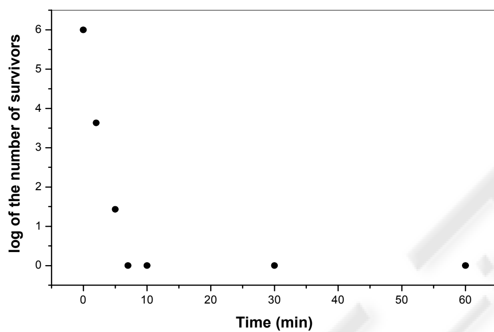
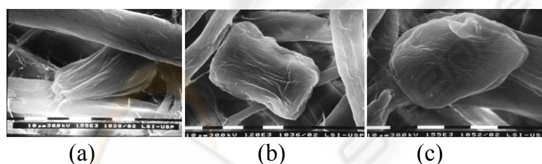


Figure 1: *B. stearothermophilus* exposed to RIE plasma at 330 mTorr pressure, 100 W power and 500 sccm O_2 flow.



(a) (b) (c)

Figure 2: (a)- Spore before exposure to a plasma process; (b)- Spore after a 10-minute exposure to the plasma process; (c)- Spore after exposure to autoclave sterilization.

Figure 3 shows the number of survivors of *B. subtilis* microorganisms (inoculated on glass plates) when exposed to the oxygen plasma process. It can be observed that, after 20 minutes of processing, there is a reasonable decrease in the number of survivors at a power of 100 W, with this number being reduced to zero after around 60 minutes. When 150 W is applied, the microbial load is rapidly reduced, being zeroed out after 20 minutes of processing.

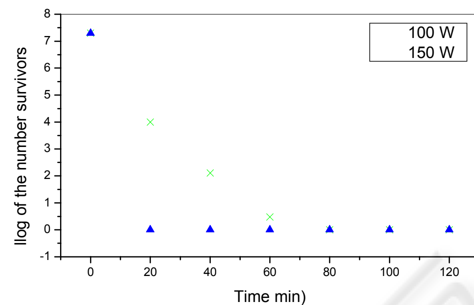
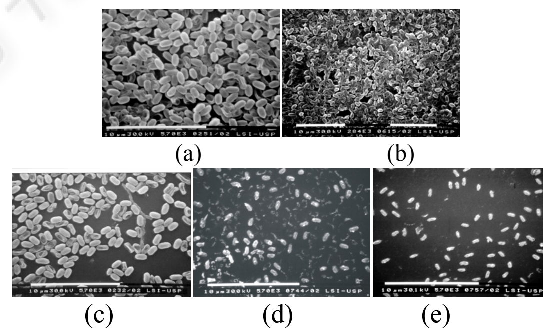


Figure 3.: Graphic of the number of survivors of the indicators *B. subtilis*, inoculated on glass plates and exposed to the plasma process for 2 to 120 minutes, under O_2 flow of 200 sccm and pressure of 100 mTorr.

Figure 4 shows a comparison of *B. subtilis* indicators before and after exposure to the plasma, X-ray and UV processes. When exposed to the X-ray and UV processes, the form of the microbes does not change when compared to it before the application of the processes. These micrographs corroborate the results of the survivor counts in which it was possible to verify the non-reduction of the microbial load. When it is used the oxygen plasma processes, deformation of the microorganisms is observed as well as spaces between them, something that does not happen in the other processes.



(a) (b)
(c) (d) (e)

Figure 4.: (a) Sample before the processes; (b) Sample after the UV process; (c) Sample after the X-ray process; (d) Samples after the plasma process with 100 W of power; (e) Sample after the plasma process with 150 W.

4 CONCLUSIONS

The system proves to be efficient for sterilization; the process is fast and does not attack the materials being sterilized. As the sterilization time is quite short, the oxygen plasma can be applied to test its

potential to sterilize cellulose materials inoculated with *B. subtilis* and *B. stearothermophillus*. The tests with materials on small glass plates suggest the utilization of this technique for the sterilization of several medical products, because of the fact that with shorter exposure times, the microbial load is reduced to zero and the sterilized material is not attacked during the process. Besides, this process is safe for the operator and the environment. Once the process is fast and conducted at room temperature, it can also be used for sterilization of polymeric materials since tests are performed before to adjust the parameters of the process to the characteristics of the material to be sterilized.

The microbial load is reduced to zero in few minutes when 150 W of power, 100 mTorr of pressure and 200 sccm of oxygen flow is applied, requiring at the most a 20 minute process for *B. subtilis*. This duration must be increased to 60 minutes when 100 W of power is applied.

For the samples of *B. stearothermophillus*, the processing time to zero out the microbial load is around 15 minutes, using in this case 100 Watts of power, 330 mTorr of pressure and 500 sccm of flow.

For the X-ray sterilization tests, 200 to 1000 W were utilized in 30 minute processes, despite the high power levels applied (when compared with the plasma processes), the logarithmic reduction of the viability of the biological indicators remains very low to indicate a sterilization effect, what discourages the application of this technique for sterilization purposes. The UV sterilization tests at 14 W/cm² and 2 to 60 minute periods did not present satisfactory results, because the microbial load was only slightly reduced, showing that the UV radiation does not contribute to the sterilization effect.

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