

# STUDY OF LOW TEMPERATURE INACTIVATION OF SPORES USING MICROWAVE EXCITED AIR PLASMA

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## Abstract:

In the present study, we investigated the inactivation characteristics and contribution of different inactivating factors generated in a low temperature and low pressure nitrogen, oxygen and air-simulated plasma for the inactivation of *Geobacillus stearothermophilus* spores. We used three optical filters i.e. thin quartz ( $\lambda > 180$  nm), lithium fluoride (LiF,  $\lambda > 120$  nm) and Pyrex glass ( $\lambda > 300$  nm) plates to identify the most efficient wavelength range. The effect of optical radiations alone was studied through placing a small isolated and evacuated chamber with spore sample inside the plasma chamber.

**Keywords:** plasma sterilization, low temperature plasma, microwave plasma, air plasma, VUV, radicals.

## 1. Introduction

The low temperature plasma processing offers a potential approach to sterilize the exposed as well as wrapped medical instruments, because many medical instruments and the wrapping materials are made of polymers. Such materials can be easily damaged by thermal treatments i.e. dry heat, steam autoclave techniques. Other low temperature techniques such as ethylene oxide (EtO) and gamma irradiation etc. pose the formation of toxic by-products and material degradation. The plasma sterilization technique overcomes many inherent limitations of the conventional techniques through low temperature treatment of the heat-sensitive objects, short treatment time, safe operation and no toxicity after processing etc. There are several mechanisms which may be responsible for the sterilization: interaction of UV radiation with the DNA of the cell (below  $\lambda = 275$  nm), the photons have enough energy to break bonds of C-C (3.8 eV) or C-H (4.5 eV) in a solid and are known to be able to induce strand breaks in the deoxyribonucleic acid [1], removal of the material of the cells by reactive species (oxygen atoms) and the interaction of these two mechanisms [2]. In our previous work, a six-log reduction in spores could be achieved only several minutes irradiation with a low-pressure oxygen/air simulated surface-wave plasma and the chemical etching reaction from the reactive oxygen radicals and UV emission from excited nitrogen atom and molecules make more efficient inactivation rate [3]-[5]. In this work, we follow up to previous study, in order to investigate the contribution of various effecters special VUV/UV radiation in the inactivation of bacterial cells by plasma.

## 2. Experimental

The experimental setup used for the sterilization tests consists of a stainless steel cylindrical vacuum chamber having a diameter of 400 mm and a height of 400 mm with a microwave launcher fed from a 2.45 GHz microwave generator, as shown in Fig. 1 [5]. The launched microwave power varied from 0.2-3 kW. A small chamber of 150 mm in length and 70 mm in width was placed inside the processing part of main experimental setup. The top of small chamber was covered with a filter which is 30 mm in diameter and 1.5 mm of thickness to stop the radicals and let the VUV/UV go through. As already mentioned, plasma emits electromagnetic radiation ranging from far ultraviolet to the infrared, and the far-UV ( $100 \text{ nm} < \lambda < 200 \text{ nm}$ ) is very important component of VUV emission from plasma, since these photons have energy which greatly exceeds that of all chemical bonds in organic molecules.

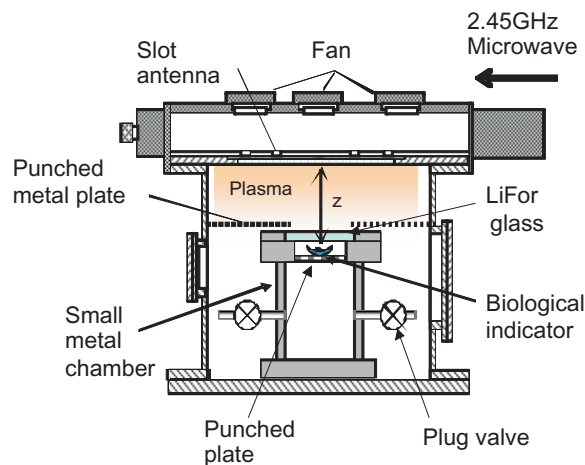


Fig. 1. Microwave excited surface-wave plasma device [5].

So, the LiF filter was used to get the emission spectra above 120 nm. The Biological Indicators (BI, for short, *Geobacillus stearothermophilus* spores with a population of  $2.5 \times 10^6$  on a small stainless disc) was put in small chamber and treated with surface-wave plasma (200 sccm flow rate at 10 Pa pressure and 700 W microwave power with on/off time as 20/60 sec). The substrate stage is about 15 cm below the quartz window. Two different pressures in the small chamber were compared each other, one is opening the plug valve to make the same pressure as surrounding condition. The other is closing the plug valve and pumping down about  $10^{-3}$  Pa in order to prevent any other plasma producing radical in the small chamber and the gas absorption for UV photons. After plasma treatment, the spores were removed from the stainless disc and putting them into culture tubes. They

were transferred to Petri dish and incubated at 50-55°C for 48 h. In order to clarify and better understanding the mechanism of sterilization, we use optical emission spectrometry to evaluate the characteristic of plasma under filter and SEM to analyze the changing of spores shape.

### 3. Results and discussions

For plasma diagnostics, optical emission spectroscopy (OES) measurements were performed using the VUV to IR monochromator systems VM-502 (Acton Research Corporation) to measure the vacuum ultraviolet part of the spectrum in the wavelength range from 100 nm to 500 nm. The MgF<sub>2</sub> window was used as the vacuum window in the port between the vacuum chamber and OES device. Figure 2 shows VUV/UV and visible light emission spectra from pure oxygen plasma at a power of 700 W and pressure of 10 Pa used in sterilization experiment. The most significant oxygen lines of these spectrum are the 130.5 nm line which correspond to the transition 3s 3S--2p4 3P [6] and 777.2 nm line which correspond to the transition 3p5p--3s3S [7]. From the Fig. 3, there were three main regions of high efficacy in the case of air-simulated gas plasma, one was the radiation from 200 nm to 250 nm wavelength range was identified to be NO- $\gamma$  radiation, from 300 nm to 400 nm wavelength range was identified to be N<sub>2</sub> second positive system, and near the VUV range there was an important Nitrogen line that was around 120 nm (transition 3s 4P--2p3 4S). The short wavelength VUV/UV penetrates into the protective layer of the microorganisms, and destroys DNA [8]. But the longer wavelength region is comparably in-effective than the shorter wavelength UV. It should be noted that the longer wavelength emission shows smaller antibacterial effect [9].

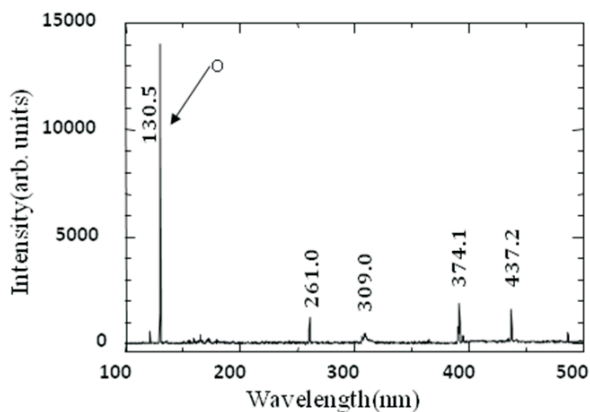


Fig. 2. Emission spectrum of pure oxygen plasma at 700 W microwave power and 10 Pa pressure.

Figure 4 shows the colony count results of the *Geobacillus stearothermophilus* spores irradiated with pure oxygen plasma and air simulated plasma. As shown in the Fig. 4, the case of direct plasma exposure provides survival plots with 3 different linear segments. After 2-min treatment, only 1% *Geobacillus stearothermophilus* colony was observed and there was no colonies growing on the agar plate at all after 5-min treatment time in the air simulated plasma and 10-min treatment time in the pure oxygen plasma. From the second linear segment, it took a longer time for inactivating the rest 1% survivors, that

because some spores stacked together and formed a multilayered structure as protected layer for UV and radicals.

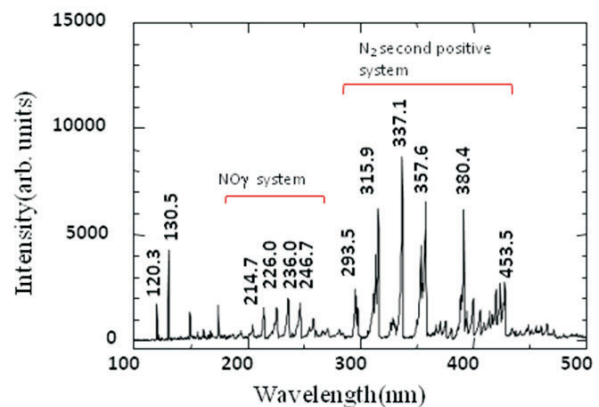


Fig. 3. Emission spectrum of air simulated plasma at 700 W microwave power and 10 Pa pressure.

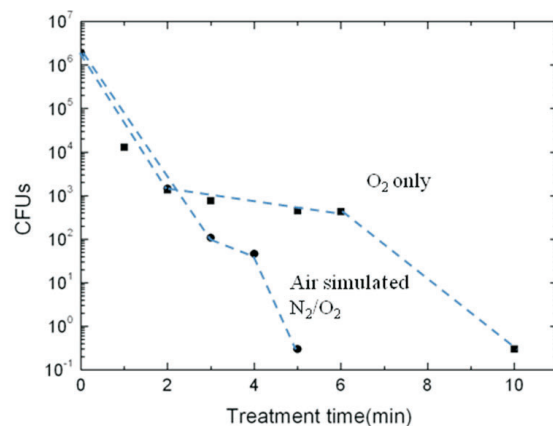


Fig. 4. Survival curves of *Geobacillus stearothermophilus* spores by oxygen plasma and air simulated plasma at 700 W microwave power.

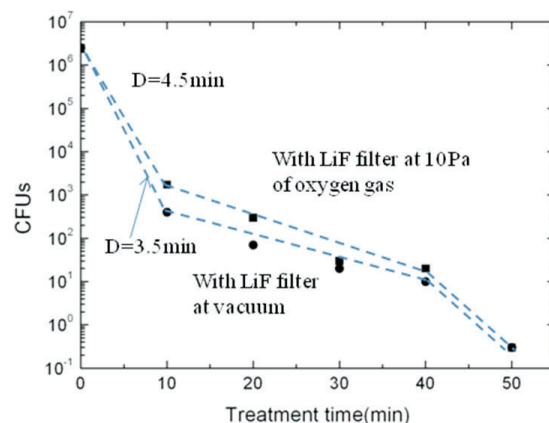


Fig. 5. Survival curves of *Geobacillus stearothermophilus* spores by oxygen plasma with a LiF filter at 700 W microwave power.

The experimental results show that the sterilizing efficiency of air simulated plasma is better than oxygen plasma. Previous studies about plasma emission spectra indicated that the effect of UV emission in air simulated plasma under identical electrical discharge conditions is stronger than that in oxygen plasma, because there were NO- $\gamma$  system UV emission, N<sub>2</sub> second positive system UV emission and VUV emission due to nitrogen atom in air simulated plasma besides VUV due to oxygen atom. VUV/UV

acts synergistically with the reactive radicals and accelerate the elimination rate of microorganisms.

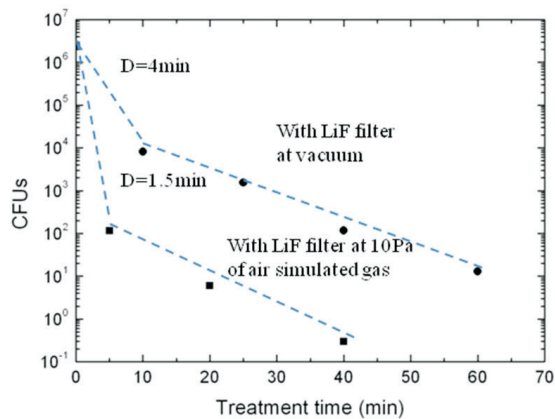


Fig. 6. Survival curves of *Geobacillus stearothermophilus* spores by air simulated plasma with a LiF filter at 700 W microwave power.

The effects of VUV/UV radiation emitted from the plasma results were shown in Fig. 5 and Fig. 6. In Fig. 5, the colony forming units of the BIs, which were set inside the small chamber with a LiF filter at different pressure conditions, were compared as a function of oxygen plasma treatment time. We calculated the D value of the first segment of survival curves as approximately 4.5 min and 3.5 min. After 50 min treatment time, there were no colonies growing on the agar plate at all under a LiF filter. Compared with the pressure effect on VUV/UV sterilization with LiF filter, we can find that the vacuum condition was more efficient than the low-pressure condition, because in the low-pressure case, gas molecules can absorb VUV/UV photons and reducing the intensity of VUV/UV reaching the BIs. In Fig. 6, the number of colony forming units for air simulated plasma case was shown. In this case, the low-pressure condition was more efficient than the vacuum condition and the number of survivors decreased by more than two orders of magnitude. In the vacuum case, only VUV/UV emission contribute to the sterilization, on the other hand, in low-pressure case, there were some radicals produced by VUV inside the small chamber and their synergistic action to the spore mortality causes an additional role in inactivation of spores.

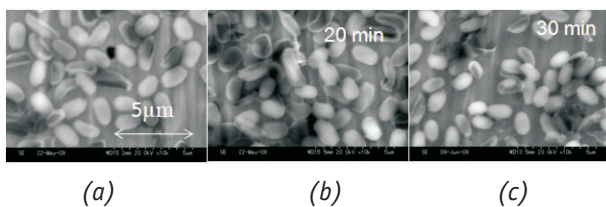


Fig. 7. SEM images of (a) untreated *Geobacillus stearothermophilus* spores and those irradiated by oxygen plasma with LiF filter for (b) 20 min in vacuum and (c) 30 min in O<sub>2</sub> gas.

We also carried out the SEM analyses of spores treated by the plasma to investigate the effects on the change of spores shape by VUV/UV radiation. Figure 7 shows the SEM images of the spores before and after pure oxygen plasma treatment with filter. In the vacuum case, there was al-

most no change of the size after 20 min treatment. It also shows that there is no significant erosion of the microorganisms when only VUV/UV radiation exists in the case of vacuum and the VUV/UV is only important factors responsible for inactivation. It is interesting to note that the spore sizes slightly decreased when oxygen gas was filled at 10 Pa in the small chamber with LiF filter, as shown in Fig. 7(c). The size of the spores decreased from original after 30 min treatment. It means there is some erosion of the microorganism due to the oxygen radicals excited by the VUV emission.

#### 4. Conclusion

In this research, we discussed the effects of VUV/UV radiation and radicals on low-temperature sterilization in surface-wave plasma. The results showed that the VUV/UV photons act synergistically with the radicals make more efficient inactivation of the *Geobacillus stearothermophilus* spores by air simulated plasma directly. We also tested the inactivation experiment depending on the VUV/UV radiation in two different pressures to evaluate the role of VUV/UV radiation in plasma based CFUs and SEM analysis. From the present study, it was concluded that The VUV/UV emission plays an important role for sterilization and there is no erosion of the microorganisms when only VUV/UV radiation exists.

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