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Effects of VUV/UV Emission and Radicals on Inactivation of Spore Forming Bacteria by Surface-wave Excited Nitrogen/Oxygen Plasma

表面波励起窒素・酸素プラズマを用いた芽胞形成菌不活化における VUV/UV放射およびラジカルの効果

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In general, under plasma exposure, bacterial cells can be destroyed by synergistic reaction of UV radiation, plasma particles and so on. Insight into each factor how to effect on bacterial and the processing are the goal of this study. In this work, we focus on the role of radicals and VUV/UV emission as a sterilization agent of *Geobacillus stearothermophilus* spores. The measurements of colony counting method and scanning electron microscope were used to investigate the effect of radicals and VUV/UV emission in detail.

1. Introduction

Currently, many sterilization techniques, for example autoclave, H_2O_2 gas sterilization and radiation sterilization are used in medical facilities. However these techniques have problems, such as high temperature, toxicity and high cost, respectively and proper use is carried out according to the purpose. And plasma sterilization mechanisms are studied to establish the low temperature plasma as a more effective sterilization tool.

Boucher et.al reported that UV photons in the plasma have an important role[1]. And Bol'shakov et.al reported that UV photons and oxygen radicals synergistically induce the chemical decomposition of microbial cell. Moisan et.al suggested that chemical reactions, UV emission, photodissociation and ion sputtering are synergistically effect, and there is the inactivation effect of spores clearly higher than the case where each acts alone[3]. And the extent of the influence of each factor depends on the plasma operating parameters such as power and gas mixture and flow rate. In our previous work, a six-log reduction in spores could be achieved only several minutes irradiation with low-pressure oxygen/air simulated surface-wave plasmas and the chemical etching reaction from the reactive oxygen radicals make more efficient inactivation rate, and in oxygen/nitrogen mixture plasma, the best efficiency of etching was appeared in the case of 90% oxygen mixture with 10% nitrogen[4-6]. But we don't know how VUV/UV damages the DNA in the spore and what relation between nitrogen radicals and the inactivation of spores in our previous works. In this work, we aimed to investigate effects of VUV/UV emission to DNA and nitrogen radicals on inactivation of spore forming bacteria.

2. Experimental setup

The experimental setup used for the sterilization tests consists of a stainless steel cylindrical vacuum chamber having a diameter of 300 mm and a height of 300 mm with a microwave launcher and 2.45 GHz microwave generator, as shown in Fig. 1. The plasma is produced at a pressure of 13 Pa and a total gas flow of 100 sccm by a microwave power source. The launched microwave power varied from 200 to 800 W. The optical emission spectroscopy was used to diagnose the plasma parameters. We used the biological indicator (BI) of *G. stearothermophilus* spores with a population of 2.1×10^6 pasted on a small stainless disc, which was put on the sample stage and treated with oxygen plasma or nitrogen plasma. The sample stage was about 10 cm below the quartz window. After treatment, one set of treated samples were washed with 2 ml of BHI (Brain-Heart Infusion) solution in the test tube for colony counts. Test tubes containing the biological indicator carriers were stirred for 1 min at room temperature. After an appropriate dilution, 0.1 ml of the spore suspension was inoculated onto nutrient agar media and after two days of incubation at 55 ºC, the colony forming units (CFUs) corresponding to each surviving cell,

were counted. The CFUs reduction of treated samples were compared with untreated sample and plotted as the survival curve. In parallel, a scanning electron microscope was used to observe the morphologies of the bacteria before and after the plasma treatment.

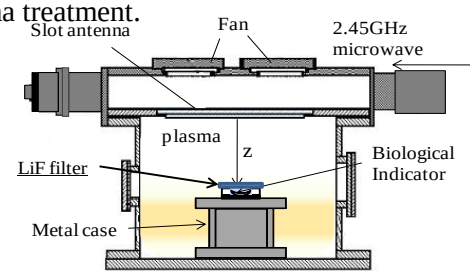

Fig.1 Schematic view of experimental setup of the surface-wave plasma device.

3. Results and Discussion

Figure 2 is the result of CFUs of *Geobacillus stearothermophilus* spores treated by oxygen plasma without and with LiF filter to block particles, such as radicals, at 400 W, 600 W, and 800 W, respectively. When the LiF filter was set above the B.I. samples, the inactivation efficiency was obviously decrease. This result might suggest that the main inactivation factor is oxygen radicals in oxygen plasma.

Figure 3(top) is the survival curves of spores treated by the direct irradiation of nitrogen plasma at 400 W, 600 W, and 800 W, respectively. And Fig.3(bottom) is the result of the survival curve when the plasma particles is intercepted by the LiF filter which cutoff wavelength is 120 nm in the same plasma condition. The differences in survival curves obtained at each condition were less than that in the case of oxygen plasma. That result means the dominate factor of the deactivation is clearly different from oxygen plasma treatment. In the case of nitrogen plasma, the dependence of plasma particles on the deactivation is much less. The main sterilization mechanism is VUV/UV radiation. However, when the microwave power was 800 W, six-log reduction time shown in Fig. 3(top) was shorter than that at 400 or 600 W. We will investigate the effect on spores only by radicals (neutrals) and VUV/UV radiation without any charged particles by inserting the grounded porous metal electrode between plasma and sample surfaces. And it will become possible to investigate the effect of radicals by the existence of a LiF filter.

4. Conclusion

In this research, we studied the relation between the effects of radicals and VUV/UV radiation and the inactivation of spores in surface-wave plasma. The other results will be shown in the further report.

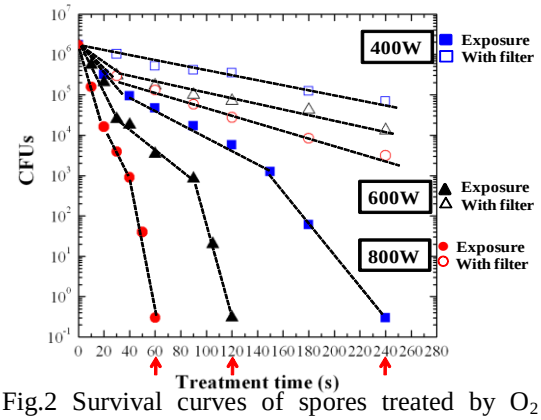

Fig.2 Survival curves of spores treated by O_2 plasma without and with a LiF filter

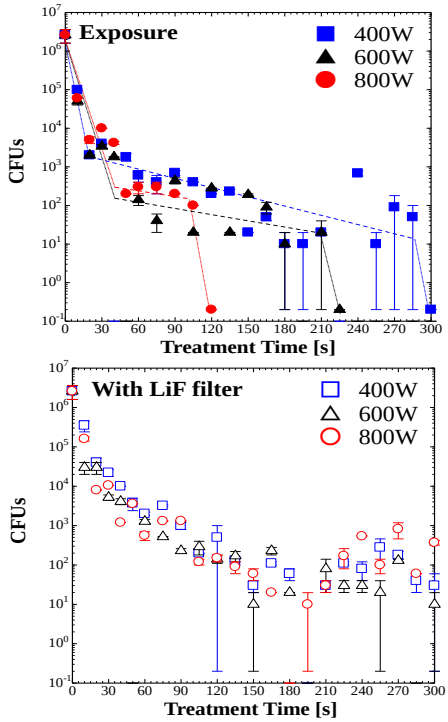

Fig.3 Survival curves of spores treated by N_2 plasma without (top) and with (bottom) a LiF filter

Acknowledgments

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