

## Effects of Oxygen and Nitrogen Atoms in N<sub>2</sub>/O<sub>2</sub> Surface-wave Plasma on Inactivation of Spore-forming Microorganisms

窒素/酸素混合ガス表面波プラズマによる芽胞菌不活化における励起酸素、窒素原子の効果

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To figure out the plasma inactivation mechanism of spore-forming microorganisms is important to improve the plasma sterilization property. In this study the effect of N and O atoms on inactivation of spores was studied by measuring the absolute atomic density using vacuum ultraviolet absorption spectroscopy (VUVAS) method. Recently, we have successfully carried out the measurement of O density in N<sub>2</sub>/O<sub>2</sub> plasma using the VUVAS method, and found a strong correlation between the oxygen atomic density and the plasma etching on spores. With the same light source, the absolute N atomic density will be measured for the next step.

### 1. Introduction

To figure out the plasma inactivation mechanism of spore-forming microorganisms is important to improve the sterilization property. But so far, the mechanism of low temperature plasma sterilization has not yet been completely understood. In our previous work, N<sub>2</sub> / O<sub>2</sub> gas mixture surface-wave plasma (SWP) generated in a 30 cm diameter SUS cylindrical chamber was used for the low-temperature plasma sterilization of *Geobacillus stearothermophilus* spores. The result showed that the UV radiation is the important lethal factor, but the neutral species, such as excited O and N atoms, also play a role in the inactivation of spores [1, 2]. However, their behaviors have been not studied enough, because only a few reliable measurement techniques for the absolute densities of radicals in reactive plasmas have been developed.

Spectroscopy measurement does little influence to the processing plasma. Among different spectroscopy diagnosis methods, only the resonant absorption spectroscopy can directly determine the absolute density of reactive species, and it had been widely used for the determination of the absolute radical density in processing plasma. In this study, surface-wave plasma produced with N<sub>2</sub>/O<sub>2</sub> mixture gas was used for investigating the mechanism of the inactivation of *Geobacillus stearothermophilus* spores. The effect of O and N atomic species on spore-forming microorganisms was investigated by using VUVAS technique with a compact coaxial microwave plasma light source.

### 2. Experimental setup

The experimental setup consists of a stainless steel cylindrical vacuum chamber (300 mm in diameter and 300 mm in height) with a microwave launcher and 2.45 GHz microwave generator and a compact microwave light source was shown in Fig.1. The light source was installed on one side (10 cm away from the top) of the chamber and the VUV monochromator (Acton Research Corp., VM-502) was fixed at the opposite port. Two MgF<sub>2</sub> glass windows were inserted to separate it into three different pressure areas. Inside the chamber, the absorption length *L* is constricted to about 30 cm.

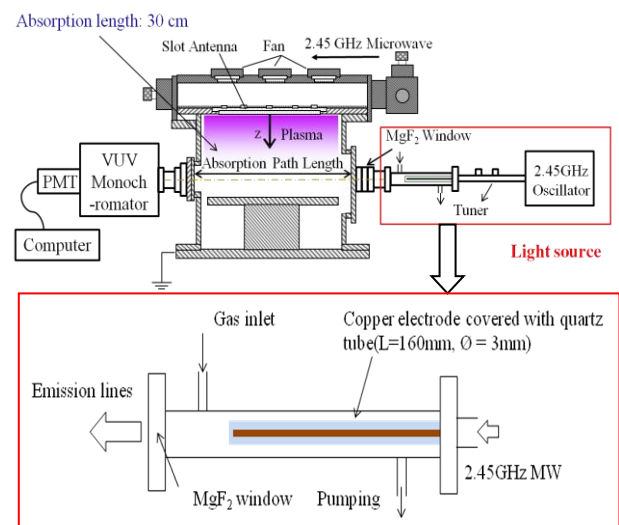


Fig.1. Schematic drawing of the experiment setup.

The pressures both in the monochromator and processing chamber could be kept on  $10^{-5}$  Torr by separated two-stage differential pumping systems.

The resonant lines used for O and N atom density measurements with VUVAS method were near 130 nm and 120 nm, respectively. In this work, we have selected Ar and a little amount of  $O_2$  mixture as the working gas for producing 130 nm oxygen transition lines and He and  $N_2$  mixture gas to produce 120 nm emission lines.

### 3. Results and discussion

With resonance emission line at 130.22 nm as a light probe, the self-absorption calibration based on escape factor theory [3], and VUVAS calculation theory [4], the absolute O atom density was measured with different gas mixture ratio of  $N_2$  and  $O_2$  in SWP. The incident power of the SWP was kept at 600 W, and the pressure was controlled at 0.1 Torr with a total flow rate of 100 sccm, the result was shown in Fig. 2.

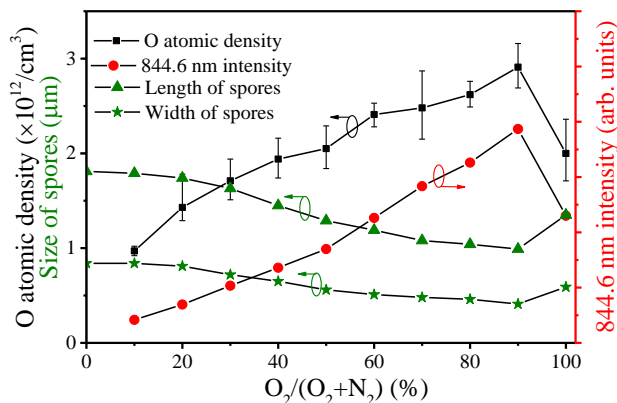


Fig. 2. Absolute O atom density, spore size and 844.6 nm emission line intensity as a function of  $O_2$  mixture ratio.

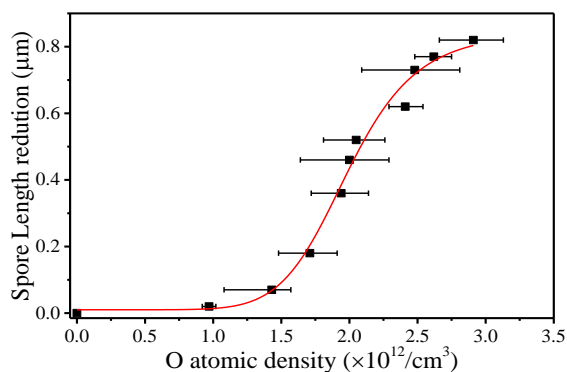


Fig.3. Relationship between O atomic density and spore reduction length due to etching

From the result presented in Fig. 2, the O atomic densities were varying from  $0.97 \times 10^{12}$  to  $2.91 \times 10^{12} cm^{-3}$  with different  $O_2$  proportion and got a biggest density when 10 % percent  $N_2$  was added. The trend of absolute O density change was consistent with

the 844.6 nm emission intensity and the sizes of spore length and width measured after 1 min plasma treatment under the corresponding conditions [5]. As shown in Fig. 3, where the spore length reduction was plotted as a function of the O atom densities, we found that when the O density is higher than  $1.7 \times 10^{12} cm^{-3}$ , the spore length reduction increased quickly with O density increasing. It means that a threshold O density exists for effective etching. But when the O density became higher than  $2.48 \times 10^{12} cm^{-3}$ , the etching rate became slowly again. When the etching reached to the dense cortex inside the spore, the etching became very slow. From these results, it can be deduced that the spore etching effect in  $N_2/O_2$  gas mixture plasma are strongly related with the O atom density.

For the N atomic density measured using the same light source with He/ $N_2$  gas and similar VUVAS method, we are now investigating the operating condition of the light source to get the optimal emission intensities and minimal self-absorption effect at about 120 nm. With the best light source condition, the N atomic density will be measured with different gas ratio of  $N_2$  and  $O_2$  in SWP to investigate its effect on the inactivation of spores.

### 4. Summary

The effect of oxygen atoms on spores had been studied with VUVAS method, the result showed the strong correlation between the spore etching and oxygen atomic density in  $N_2/O_2$  gas mixture plasma. With the similar method, the N atomic density will be measured for the next step. At the conference, we will present the experimental results of N and O atom measurement result and discuss their effects on inactivation of spore-forming microorganisms in detail.

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