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Effects of Oxygen Concentration and Water Vapor from Agar on Sterilization of Spore Using Plasma

芽胞菌のプラズマ殺菌における酸素濃度及び寒天培地の蒸散水蒸気の影響

Kenta Yonetamari, Masayuki Komatsu and Ryo Ono
米玉利健太，小松真之，小野亮

Department of Advanced Energy, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo,Japan
東京大学　〒113-8656　東京都文京区本郷7-3-1

The effects of oxygen concentration and water vapor evaporated from agar medium on plasma sterilization of spore are examined. A nanosecond pulsed streamer discharge is used. The sterilization rate increases with decreased N_2 gas flow rate. It indicates that reactive species produced from water vapor evaporated from the agar medium, such as OH and H_2O_2, sterilizes the spore because the humidity on the agar medium due to the evaporated water from the agar medium increases with the decreased N_2 gas flow rate. The spore is also sterilized by O_3. The sterilization area by O_3 is much wider than that by the reactive species produced from H_2O.

1. Introduction

Recently, cold-plasma sterilization is noticed to solve problems in conventional sterilization methods. Cold-plasma can sterilize at room temperature and be an applied to plastic materials and living organisms. Although there are various sterilization factors in plasma, reactive species are considered to have major contribution[1]. However, its mechanism has not been revealed yet. Especially, there are few discussions about that which bacteria are inactivated by which reactive species and how doses are needed for sterilization.

Our group has investigated reactive species which play an important part on plasma sterilization. In our previous research, we measured OH, O and NO radical density in nanosecond pulsed streamer discharge by laser induced fluorescence method (LIF)[2]. Based on this research, we examine in the present paper the effects of oxygen concentration and water vapor from agar medium on plasma sterilization on spore on agar medium (*Bacillus atrophaeus*, ATCC9372).

2. Experiment

2-1. Nanosecond pulsed streamer plasma

Schematic diagram of nanosecond pulsed streamer discharge is shown in Fig. 1. The rod electrode of 3 mm diameter is made of copper. Its end is formed into a semispherical shape of 1.5 mm curvature. The electrode is inserted into a quartz tube (inner diameter :4 mm, thickness :1 mm) concentrically, so any gas can be introduced. Using a nanosecond pulsed power supply, this equipment can generate stable streamer discharge.

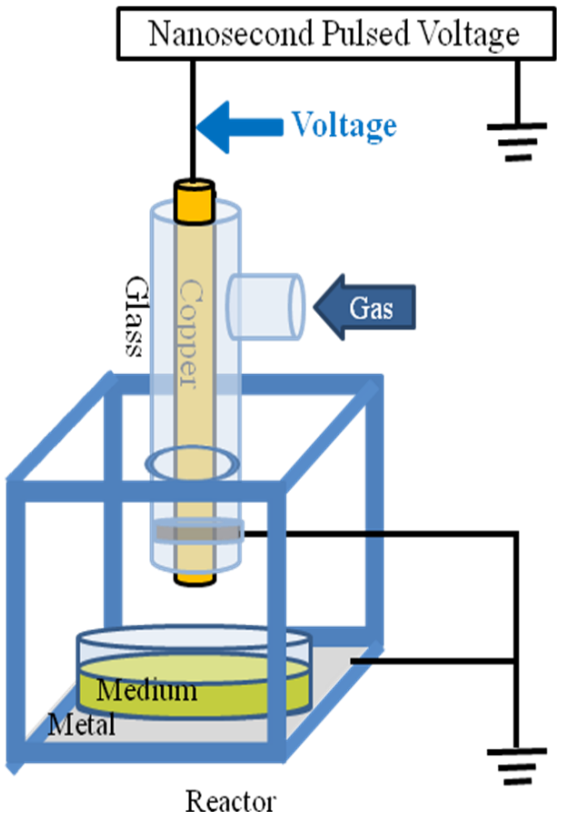

Fig.1 Schematic diagram of nanosecond pulsed streamer plasma

2-2. Sterilization

In this study, *Bacillus atrophaeus* (ATCC 9372) which is a kind of spore was used as a target of sterilization.

Spore can survive whichever both moist air like on agar medium and drying atmosphere like on stainless. In this experiment, we applied plasma to spore on agar medium.

3. Results

3-1.Effect of gas flow rate on sterilization

In this experiment, N_2 gas flowed at 0.1 L/min and 1 L/min. The electrode gap distance was 5 mm and the irradiation time was 10, 30, 90, 120, 180, 300, and 600 seconds. As a control, 120 seconds of N_2 gas flow without discharge was prepared.

Fig. 1 shows sterilization results for different N_2 flow rates. When the irradiation time was 120 seconds, sterilization with 0.1 L/min gas flow was more effective than that 1 L/min gas flow. On the other hand, at 600 seconds, sterilization area with 1 L/min gas flow was wider than that with 0.1 L/min gas flow. Besides, for both gas flow rates, as the irradiation time was longer, the sterilization area was wider.

These results indicate that under N_2 gas conditions, radicals produced from water vapor are effective on sterilization, for example, OH, HO_2, H_2O_2. More radicals are produced in the vicinity of agar medium surface by reducing gas flow rate.

3-2. Effect of O_2 concentration on sterilization effects of mixing ratio

In order to change concentration of reactive oxygen species (ROS), N_2/O_2 gas was used with different O_2 concentration (N_2:O_2 = 100:0, 80:20, and 20:80). The electrode gap distance was also 5 mm and the irradiation time was 120 seconds. As well as former experiment, the gas flowed at 0.1 L/min or 1 L/min.

Sterilization results for different O_2 concentrations are shown in Fig. 2. From this experiment results, as O_2 concentration is higher, sterilization effect is stronger on a wide area of the medium. It is assumed that increased ROS produced from O_2 caused the wide area of sterilization. The wide sterilization area indicates that the sterilization was caused by O_3 of long life ROS.

Paying attention to sterilization effect on the center of medium, sterilization was effective in 0.1 L/min gas flow and 0 % O_2 concentration condition. It was observed that an increase of O_2 concentration decreases the density of OH radicals[2], therefore this result suggests that OH radical is effective on sterilization.

4. Conclusions

In plasma sterilization of spore on agar medium, it was suggested that sterilization effects of O_3 and radicals originated from humidity are strong. At present, there are rarely studies that examine the effect on sterilization of water vapor from medium surface, so research related with this effect is needed in the future.

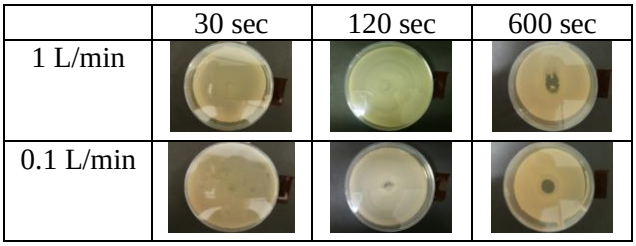

Fig. 1 Sterilization results for different N_2 flow rates

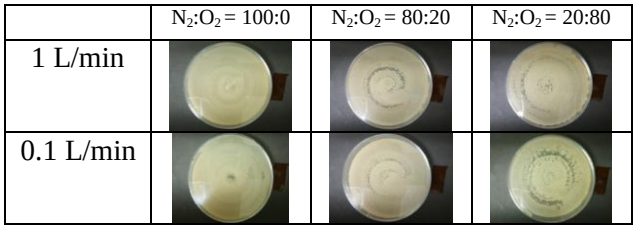

Fig. 2 Sterilization results for different O_2 concentrations

References

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