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Study of Microbial Sterilization by Atmospheric Remote Microplasma

S. Tatematsu, N. masamura, M. Blajan and K. Shimizu

Organization for Innovation and Social Collaboration, Shizuoka University

Jouoku, Hamamatsu, 432-8561, Japan

E-mail : shimizu@cjr.shizuoka.ac.jp

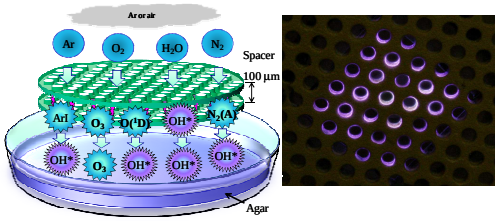
INTRODUCTION

An effective and economical sterilization method is required in the recent years. Therefore plasma sterilization received a lot of attention and intensively studied as a replacement of traditional sterilization methods. In addition, "Plasma medicine" is got a lot of attention as new application field of plasma technology.

Microplasma is studied in our group is atmospheric non thermal plasma which can be generated relatively low discharge voltage. It is a kind of dielectric barrier discharge and discharge gap is in the order of micro meters. Remote sterilization of surface-associated bacteria by atmospheric microplasma was investigated.

METHODS

(1)Sterilization process and electrodes



(a) Sterilization process (b) Image of microplasma

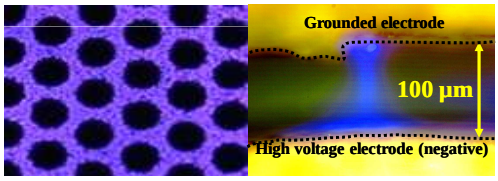
Fig. 1 Sterilization process for *E. coli* and image of microplasma during discharge.

Fig. 1 shows the electrodes and sterilization process. Electrodes are perforated metal plates covered with a dielectric layer and faced together. Microplasma was generated between the electrodes by the applied voltage thus active species and radicals were produced according to gas composition. Table 1 describes the detail of electrodes.

Table 1 The detail of electrodes.

Diameter	45 mm
Hole diameter	1.6 mm
Aperture ratio	30%
Discharge gap	100 μm
Discharge area	100 mm^2
Gas flow rate	10 L/min

(2) Image of streamer of discharge



(a) Electrode surface (b) Streamer from discharge

Fig. 2 Image of a streamer from microplasma discharge.

Microplasma is typically dielectric barrier discharge. The image of emitting of streamer is shown in fig. 2. Streamer diameter was thinner than previously reported.

(3) Experimental Setup

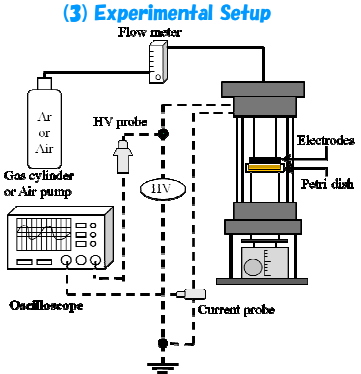
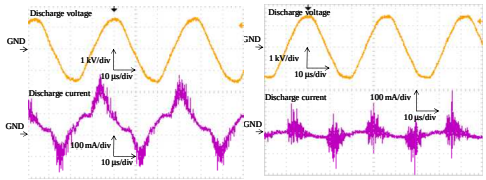


Fig. 3 Experimental setup

Fig. 3 shows experimental setup for sterilization of *E. coli*. *E. coli* was diluted in the liquid medium and the culture medium was applied on the agar medium. Then the agar medium was inserted in the microplasma reactor and exposed to remote microplasma. The distance between agar medium and electrodes could be adjusted by Z-axis stage.

RESULTS

(1)Electric Characteristics

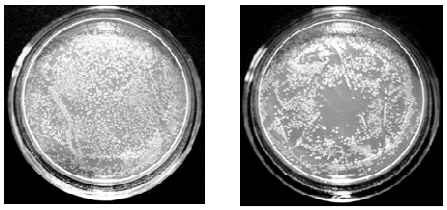


(a) Ar microplasma (b) Air microplasma

Fig. 4 current-voltage characteristic

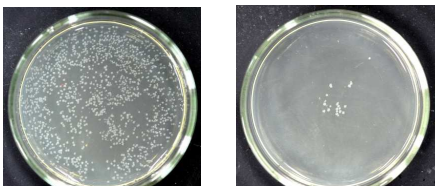
Spike-like currents occurred at the steepest slopes of the discharge voltage. This is a typical waveform of dielectric barrier discharge shown in Fig. 4.

(2) Sterilization result



(a) No treatment (b)Ar microplasma 850 V

Fig. 5 The experiment result of sterilization by using Ar microplasma. Treatment time was 60 s, distance between electrodes and *E. coli* was 2 mm.



(a) No treatment (b)Air microplasma 1.5 kV

Fig. 6 The experiment result of sterilization by using air microplasma. Treatment time was 60 s, distance between electrodes and *E. coli* was 2 mm.

Figs. 5 and 6 show the sterilization result of *E. coli*. Ar microplasma treatment could sterilize near the center of Petri dish. However, air microplasma treatment could sterilize more widely area than Ar one. This could be due to the long lifetime active species derived from O_2.

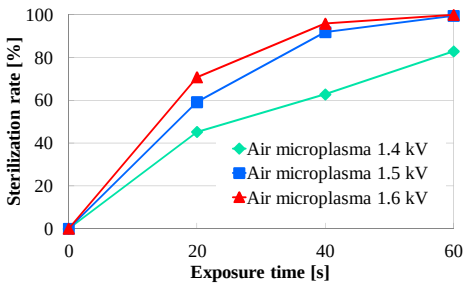
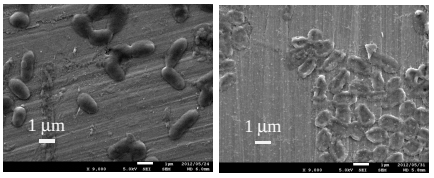


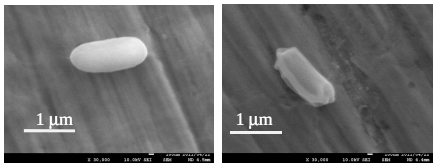
Fig. 7 The experiment result of sterilization rate by using air microplasma. Treatment time was 0 - 60 s, distance between electrodes and *E. coli* was 2 mm.

Fig. 7 shows the sterilization rate by air microplasma. The culture solution of *E. coli* was diluted to 10^4 and applied on the agar medium due to make the colonies. The colonies was counted after microplasma exposure and sterilization rate was calculated.



(a) Before treatment (b) Ar microplasma 650 V

Fig. 8 Image of *E. coli* before and after Ar microplasma treatment(x9,000, treatment time 60 s).



(a) Before treatment (b) Ar microplasma 650 V

Fig. 9 Image of *Alicyclobacillus* before and after Ar microplasma treatment(x30,000, treatment time 60 s).

Fig. 8 shows the image of *E. coli* which was the nursing cell state and Fig. 9 shows the image of gram-positive bacteria *A. acidoterrestris* was spore state taken by SEM. *E. coli* and *A. acidoterrestris* were physically damaged by applying microplasma as shown in Figs. 8 and 9 (b). This could be considered that active species derived from oxygen e. g. OH radicals exert sterilization effect.

(3) Electron spin resonance method

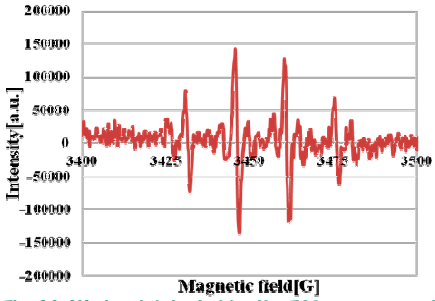


Fig. 10 OH signal detected by the ESR measurement (treatment time 3 min.).

Spin trap agent 5,5-dimethyl-1-pyrroline N-oxide was used to perform ESR analysis. Generated OH radical by microplasma were observed at 3445, 3460, 3430, 3475 gauss.

In water these active were generated during discharge via reactions (1), (2), and (3)[1].

$$O\cdot + H_2O \rightarrow \cdot OH + \cdot OH \quad (1)$$

$$e^{-}\cdot + H_2O \rightarrow \cdot OH + \cdot H + e^{-} \quad (2)$$

$$\cdot OH + \cdot OH \rightarrow H_2O_2 \quad (3)$$

Among this active species and radicals, OH and O_3 could play an important role in the sterilization process of bacteria.

CONCLUSIONS

Microplasma generated at atmospheric pressure and low discharge voltage was used for sterilization and measured surface potential. The following conclusions were obtained by the series of experiment.

(1)Air microplasma sterilized a wider area than Ar microplasma treatment. Almost 100% of sterilization rate was completed by 60 s of air microplasma treatment.

(2)The photos taken by SEM indicate that micro-plasma sterilization acts like an etching process.

(3)OH radical was one of the causes of indigo carmine decomposition. It was dissolved in the water as confirmed by using ESR.

[1].K Takahashi, Y Sasaki, S Mukaigawa and K Takaki *IEEE Trans. on Plasma Sci.* **38** 2694-2700 (2010).