

Sterilization using a wide-gap discharge under atmospheric pressure

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Abstract— Sterilization under atmospheric pressure using dielectric barrier discharge (DBD) was widely studied and it is known to be very effective. However, it is effective only when the discharge gap is very small (typically less than a few mm) because the number of streamers decreases significantly with the gap. For practical application of discharge-based sterilization, effective wide gap discharge at atmospheric pressure should be developed. In this study, combination of DBD and surface discharge was examined for generating wide gap discharge. Sterilization was examined using *Bacillus atrophaeus* (ATCC 9372) spores as a biological indicator, which is one of the most resistant bacteria. Effective sterilization was observed with the discharge gap up to 8mm with the combination of DBD and surface discharge. Sliding discharge was also investigated to generate stable and spatially homogeneous surface discharge. The sliding discharge can be generated by coupling DBD and DC high voltage application. Seeding discharge by DBD is extended toward the third electrode on which DC high voltage is applied. Sliding discharge induced by pulsed high voltage and negative DC high voltage resulted in 6 order reduction of the spores in 10min. It was also found that the effect of sterilization using the sliding discharge was affected by the surface condition of the samples.

Keywords— sterilization, dielectric barrier discharge, sliding discharge, wide gap discharge, moisture

I. INTRODUCTION

Sterilization is one of the most important processes for guaranteeing the safety of food or medical services. Discharge plasma can dissociate hydrogenperoxide (H_2O_2) into OH radicals, which are strong oxidants. Recently, OH radicals have been used for sterilization of medical instruments, etc. Since the lifetime of OH radicals are very short, the safety against leakage is high.

The sterilization system using low pressure discharge plasma to generate OH radicals has been in practical use. Sterilization using atmospheric pressure discharge plasma has also been widely studied [1-7]. All of these systems are batch treatment requiring long processing time. Sterilization of a conveyer surface, plastic film for packaging is of great concern these days, and they require atmospheric and continuous treatment.

We have studied sterilization using dielectric barrier discharge (DBD) [8] and surface discharge. Sterilization using DBD has many advantages, such as short treatment time, low temperature treatment (normally room temperature) and no use of toxic chemicals. The DBD reactor, however, requires helium or argon based gas to generate wide gap discharge at atmospheric pressure, which is not preferable for practical application. Pre-ionization using soft X-ray irradiation[9] was also studied to generate wide gap atmospheric pressure glow discharge but it is still very difficult to generate wide gap air plasma at atmospheric pressure. In our conventional DBD reactor, the effect of sterilization is high enough

with the electrode gap shorter than 2mm. Surface discharge is also one of the possible alternatives to generate stable atmospheric pressure plasma but is not widely studied for sterilization, because it is difficult to generate homogeneous large area discharge. In this study, we examined expanding the discharge gap using a surface discharge as one of the DBD electrodes. Expansion of surface discharge area was also examined by simultaneous application of negative DC high voltage and surface discharge.

II. EXPERIMENT

A. Test sample and evaluation of viability

Bacillus atrophaeus (ATCC 9372) spore, which is very resistant against dryness and heat, was used as a biological indicator or test sample. In order to evaluate sterilization performance, effect of sterilization and D-value were used. The number of colonies was counted using standard plate count after the sample was cultured. Colony forming unit (CFU) corresponds to the number of viable cells. The effect of sterilization is defined as a logarithm of the ratio of CFU before and after the sterilization treatment, as shown in equation (1). The D-value is the time required to reduce a microbial population to 1/10, which is one decimal. (equation(2))

$$\text{Effect of sterilization} = -\log_{10} \frac{CFU_{\text{treated}}}{CFU_{\text{initial}}} \quad (1)$$

$$D \text{ value} = \frac{\text{Treatment time [s]}}{\text{Effect of sterilization}} \quad (2)$$

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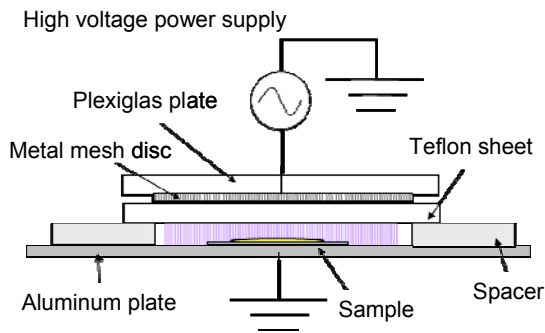


Fig. 1. Schematic illustration of the plasma sterilization reactor using DBD

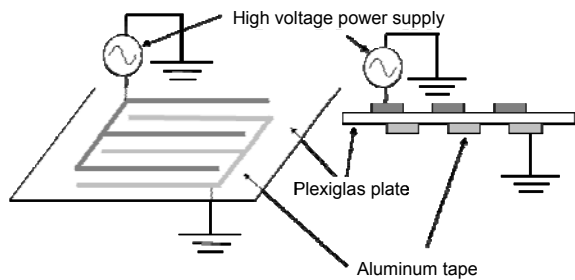


Fig. 2. Schematic illustration of the surface discharge setup

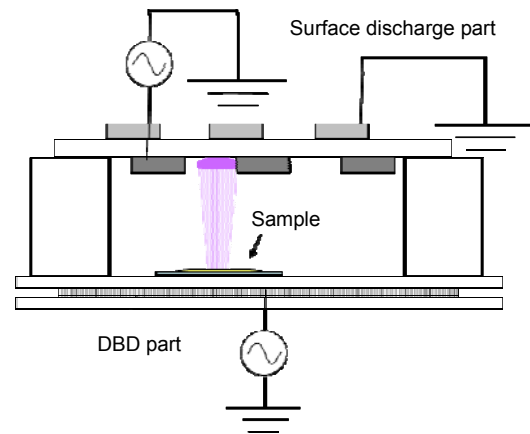


Fig. 3. Schematic illustration of the wide gap plasma sterilization reactor using DBD coupled with surface discharge

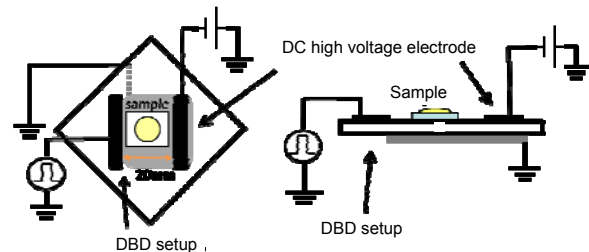


Fig. 4. Schematic illustration of a large area plasma sterilization reactor using sliding discharge

B. Experimental setup

Fig.1 illustrates a DBD setup. The DBD electrode was made of a stainless steel mesh disk with diameter of 100mm. The DBD electrode was covered with a Plexiglas plate to avoid undesirable discharge. A Teflon sheet of 2mm thickness was used as a dielectric barrier. Aluminum plate was used as a ground electrode. The DBD reactor was driven by AC high voltage ($40\text{kV}_{\text{p-p}}$), and the gap distance was 2mm. Fig.2 illustrates the surface discharge setup. High-voltage electrode was on one surface of the Plexiglas plate and the GND electrode was on the other surface. Fig.3 illustrates an experimental setup of DBD coupled with a surface discharge. Both the DBD part and surface discharge part were the same as figures 1 and 2. The distance between the DBD part and the surface discharge part was 8mm. In Fig.3, the surface discharge part was driven by AC high-voltage ($42\text{kV}_{\text{p-p}}$ 60Hz). The DBD part was driven by another AC high-voltage ($40\text{kV}_{\text{p-p}}$ 2kHz). The ambient temperature during the experiment was room temperature, about 20 degrees C. The biological sample for sterilization experiment was placed on the DBD part. Treatment time is the duration in which samples are exposed to the discharge plasma. The biological samples used in this study were prepared in the following protocol. Substrates (PET film 20mm x 20mm) were sterilized by UV lamp for 48hours. *Bacillus atrophaeus* (ATCC 9372) spore suspension with concentration between 10^7 and 10^8 CFU/cm³ was

prepared by diluting the original spore suspension. The spore suspension of 0.1ml was spotted and spread on the PET film and then dried at room temperature (20 degree C, R.H.:50%, 24hours) in a clean bench. Some samples were dried at high temperature (60 degrees C, R.H.: 7%, 24 hours) to reduce desorbed moisture on the sample surface.

Fig.4 illustrates the experimental setup of sliding discharge, which generates homogeneous wide gap surface discharge on an insulator surface [10-11]. The surface discharge electrode and DC high-voltage electrode were placed on one surface of a Teflon plate (2mm thickness), and another electrode was put on the other surface and grounded. The distance between the surface discharge electrodes and the DC high-voltage electrode was 20 mm. By applying AC high voltage to the surface discharge electrode and negative high-voltage to the DC high voltage electrode, surface discharge was extended from the AC electrode to the DC electrode and a large area discharge was generated. No extended discharge was observed with positive high-voltage application to the DC electrode; only surface discharge was observed at the fringe of the AC electrode [12-13]. The biological indicator was placed on the Teflon sheet between the AC electrode and DC electrode. The AC electrode was driven by pulse high-voltage ($24\text{kV}_{\text{p-p}}$ 2kHz). And DC high voltage (-10kV) was applied to the DC electrode.

III. RESULTS AND DISCUSSION

A. Effect of sterilization using DBD reactor

Fig.5 shows the photograph of DBD and Fig.6 shows the experimental result of sterilization using the DBD. The experimental condition was as follows: applied voltage was 40kV_{p-p} of sinusoidal wave, frequency was 1 and 2 kHz, discharge gap between the dielectric barrier (Teflon sheet) and the grounded electrode was 2mm, and treatment time was 10, 20, and 30 seconds. Spatially homogeneous and stable discharge plasma was generated in whole the gap. Viability of *Bacillus atrophaeus* spores decreased with treatment time and higher frequency resulted in higher sterilization effect suggesting that the effect is dependent on the energy consumption in this case. When the frequency of the applied voltage was 2kHz, the number of survivors decreased by 10⁻⁶ after 20 seconds DBD treatment, namely, all *Bacillus atrophaeus* spores were sterilized by DBD in 20 seconds. D-values were around 5 and 3.3 seconds in 1 kHz and 2 kHz respectively with this DBD reactor.

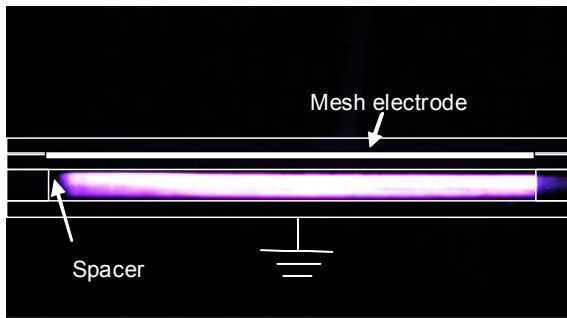


Fig. 5. Time-averaged photograph of the DBD

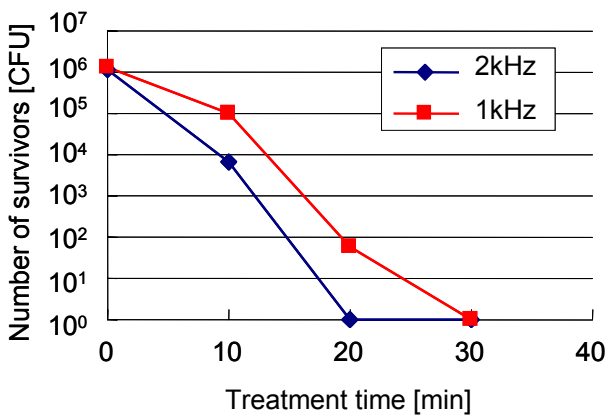


Fig. 6. Number of survivors versus treatment time using DBD

B. Effect of sterilization using DBD with surface discharge

Fig.7 shows the cross-sectional photograph of the DBD coupled with surface discharge and Fig.8 shows the experimental result of sterilization using simultaneous application of DBD and surface discharge. The applied voltages were 42kV_{p-p}, 60Hz for surface discharge and 40kV_{p-p}, 2kHz for DBD. Discharge gap was 8 mm. Stable wide gap (8mm) discharges was successfully generated. However, the volumic discharge was generated only directly under the surface discharge electrodes. The plasma was spatially less homogeneous compared with the DBD shown in Fig. 5.

The effect of sterilization obtained after 20 seconds treatment was a reduction of 6 orders indicating that it can be assumed that *Bacillus atrophaeus* spores were completely sterilized in 20 seconds in this method in spite of the imperfect uniformity of the plasma. D-value of this method was about 3.3 seconds, which was as effective as that by DBD plasma. It is notable that these results suggest a practical importance because not only flat but also bulky objects can be sterilized in this method.



Fig. 7. Time-averaged photograph of the DBD coupled with surface discharge

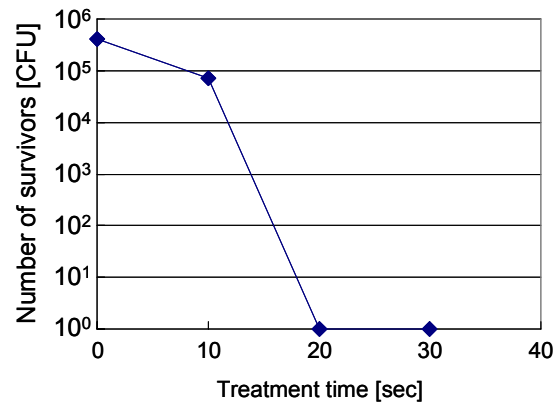


Fig. 8. Number of survivors versus treatment time using DBD coupled with surface discharge

C. Effect of sterilization using sliding discharge

Sterilization using extended surface discharge or sliding discharge was investigated. The pulsed voltage used for surface discharge generation was $24kV_p$ with positive polarity. Rise time and pulse duration of the applied voltage was $0.4\mu sec$ and $3\mu sec$ respectively. Pulse repetitive frequency was $2kHz$ in this study. DC voltage of $-10kV$ was applied to the counter electrode $20 mm$ apart from the surface discharge electrode. Back electrode was grounded. Treatment time examined were 10, 20 and 30 minutes. Fig.9 shows the time averaged photograph of the sliding discharge taken from the top of the reactor. Luminous sliding discharge between the AC and the DC electrodes as well as intense surface discharge around the AC electrode was generated. The sample was placed in the sliding discharge area.

Fig.10 shows the experimental result of the sterilization using the sliding discharge. Number of survivors decreased with time but saturated when the treatment time was longer than 20 minutes. Viability reduction by 10^{-3} and 10^{-4} were obtained by 10 and 20 minutes treatment respectively. D-values were 200 and 300 seconds respectively suggesting much lower sterilization effect than those of DBD or DBD with

surface discharge.

In order to investigate the reason why the sliding discharge showed the lower sterilization effect, sterilization using sliding discharge was examined in several different conditions. The experimental result when the sample was placed upside down (the sample surface on which the spore had been spotted was downward or facing the Teflon plate) is also shown in Fig.10. Viability reductions were only 10^{-1} at 10 min and 10^{-2} at 20 min or later. Sliding discharge can be generated on both sides of the sample in this reactor configuration and the last one. It was more intense on the top surface of the sample compared with the bottom surface judging from the results of sterilization test. Because of the discharge plasma dispersed on both sides in these reactor configuration, concentrating the discharge plasma on one surface can increase the sterilization effect.

To prevent generating the sliding discharge on the rear surface of the sample, sample was placed upside down bridging the AC and the DC high voltage electrodes. In this configuration effect of discharge dispersion can be ignored. Fig.11 illustrates the schematic illustration of the setup for this test. Thin glass cover slip ($25\times60\times0.15mm$) was used as a substrate of the biological sample in place of a PET film to prevent deformation of the sample by electrostatic force and to

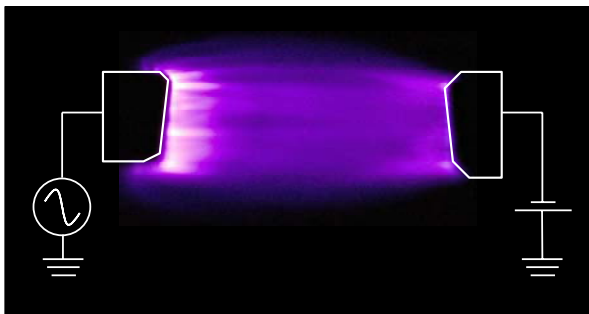


Fig. 9. Time-averaged photograph of the sliding discharge

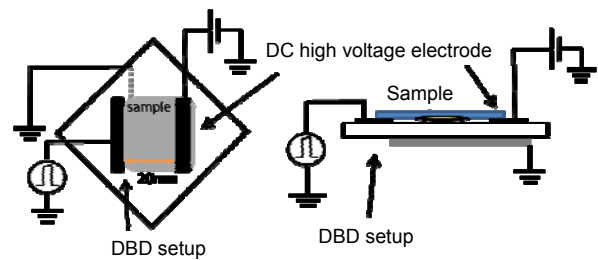


Fig. 11. Experimental setup for sterilization using sliding discharge with better exposure to the plasma

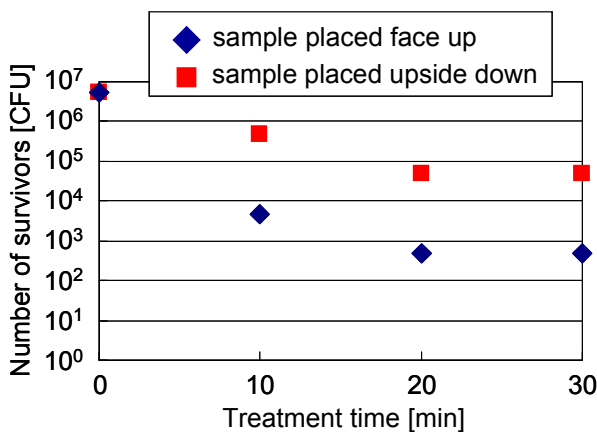


Fig. 10. Number of survivors versus treatment time using sliding discharge

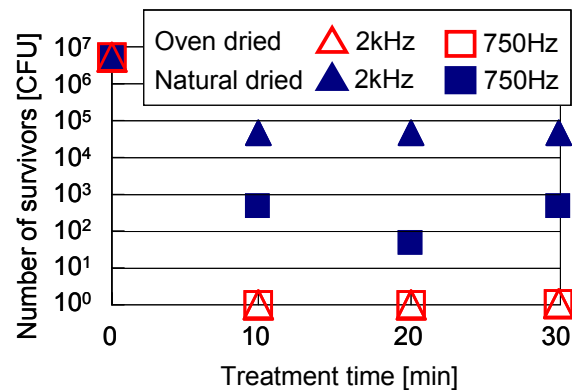


Fig. 12. Number of survivors versus treatment time using sliding discharge

keep the gap between the sample and the plasma reactor constant.

The effect of residual moisture on the sample surface was experimentally examined with above setup. Experimental condition such as applied voltages was the same as above. Fig.12 shows the result when the sample had been dried in normal way or 24h at room temperature and room humidity. Number of survivors reduced with time and saturated at 10 minutes or later. The maximum reduction of the viability was 10^{-4} to 10^{-5} . There was no significant increase in sterilization effect although the plasma was concentrated on one surface of the sample compared with the result in Fig.10. This result suggests that dispersed and weakened plasma is not an essential reason for the low sterilization performance by sliding discharge. Energy consumption by the sliding discharge plasma is proportional to the pulse repetitive frequency assuming that the energy per pulse is not dependent on the frequency. Approximately 2.6 times higher energy was consumed by the plasma when the frequency was 2kHz compared with 750Hz. However, Fig.12 shows that lower frequency led to higher sterilization effect. This result also suggests that energy consumption or intensity of the plasma does not primarily affect the sterilization effect in this case.

Figure 13 also shows the result when the sample had been dried in an oven (60 degree C, RH: 7%) for 24 hours to reduce the residual moisture on the sample surface. Sterilization test was carried out immediately after taking the sample out of the oven to prevent unintended moisture adsorption. The number of survivors decreased by 10^{-6} in 10 minute exposure to the discharge plasma, which was much more effective compared with normally dried sample. Samples prepared in this way resulted in more luminous and homogeneous discharge suggesting that moisture has negative effect on generation of the sliding discharge plasma. On the other hand, wet plasma normally results in higher sterilization effect than dry one [8] possibly through higher OH radical generation[4]. Therefore, the effect of sterilization using sliding discharge should be a trade-off between the plasma generation (uniformity or intensity) and OH radical generation, both of which are affected by moisture. These results suggest that sliding discharge is dominantly affected by the property of the sliding discharge through the surface conditions such as conductivity.

IV. CONCLUSION

DBD, wide gap DBD with surface discharge, and sliding discharge were examined at atmospheric pressure for sterilization of *Bacillus atrophaeus* spores. Following results were obtained:

1. In DBD, *Bacillus atrophaeus* (ATCC 9372) spores were completely sterilized in 20 second treatment in the most effective case.
2. Wide gap DBD up to 8 mm was generated in air at atmospheric pressure when normal DBD reactor was coupled with surface discharge.
3. The wide gap DBD up to 8 mm showed sterilization effect comparable to the normal DBD, whereas normal DBD was effective only when the gap was 2 mm or shorter.
4. Viability reduction by 10^{-6} was obtained by exposing to the sliding discharge when the sample was dried at 60 degree C and 7 %R.H. for 24 hours.
5. The effect of sterilization can be significantly affected by the residual moisture on the sample when the sliding discharge was employed.

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