Sterilization using Dielectric Barrier Discharge at Atmospheric Pressure

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Abstract—A newly developed plasma method has advantages of low temperature operation, time-saving and non-toxicity over the conventional methods, such as dry heat, steam autoclave, γ-ray irradiation and ethylene oxide (EtO) gas. Dielectric barrier discharge (DBD) can be exposed to plasma under atmospheric pressure. The effect of sterilization of Bacillus subtilis and Escherichia coli (E. coli) was investigated using DBD plasma under dry and wet conditions at atmospheric pressure. The results show that the plasma sterilization method is relatively high speed for Bacillus Subtilis spore compared with conventional methods. The wet plasma method showed higher performance compared with the dry method. These results suggest that H₂O plays an important role in the sterilization using the DBD plasma.

Keywords—discharge plasma, dielectric barrier discharge (DBD), sterilization

I. INTRODUCTION

In the food processing and medical field, sterilization is one of the most important processes for guaranteeing safety of service. Recently, OH radicals, which are strong oxidants, are used for sterilization of medical instruments, etc. Since lifetime of OH radical is very short, safety against their leakage is high. For generation of OH radical discharge plasma can be used to dissociate H₂O₂ to OH. For example, an equipment using low pressure discharge plasma in H₂O₂ vapor is already in practical use. Sterilization methods using OH radicals produced by atmospheric discharge plasmas have also been studied [1-3]. Those plasma sterilization devices, however, are operated as batch process, and evacuation is necessary in each operation. In addition, those methods need injection and decomposition of H₂O₂. Therefore, continuous operation of the plasma sterilization at atmospheric pressure has been required for variety of applications. We investigated a possibility of continuous surface sterilization using discharge plasma under atmospheric pressure without any chemicals.

Dielectric barrier discharge (DBD) is generally used to generate stable plasma at atmospheric pressure. The dielectric barrier inserted in between two parallel electrodes prevents arcing, and an AC voltage application generates non-thermal plasma [4]. In this study, we examined surface sterilization using DBD with H₂O addition. It is well expected that H₂O dissociates to OH radicals [5] in the neighborhood of bacterium. The results indicate that the DBD method is more effective compare with existing methods such as steam autoclave, ethylene oxide gas, or ozone gas system, which typically require one hour or more for one batch process.

A. Photograph of Bacillus Subtilis

Fig.1 is a phase contrast microscope (PCM) photograph of the Bacillus Subtilis used in this experiment. The particles circled in Fig.1 are the spores which are highly resistant against dryness and high temperature. Therefore we used the spore as a biological indicator in the experiment.

![Phase contrast microscope photograph of Bacillus Subtilis](image)

In order to evaluate the effect of sterilization performance, following values were used. The first one is the effect of sterilization (viability), which is defined as a logarithm of the ratio of CFU, or number of survivors, before and after the sterilization treatment as shown in equation (1). The abbreviation CFU stands for ‘colony forming unit’. The CFU value was measured using standard plate count method [1]. The second is the decimal reduction time or so-called D-value. The D-value is the time required under specified conditions to reduce a microbial population to 1/10 (decimal reduction value). In this experiment, the treatment time necessary for reduce the viability to 1/10 is used as the D-value.
Effect of sterilization = \[
\log_{10} \frac{\text{The number of population CFU before treatment}}{\text{The number of population CFU after treatment}} \quad (1)
\]

\[D - \text{value} = \text{Treatment time (sec) necessary to reduce the viability to 1/10} \quad (2)\]

B. Experimental Apparatus
Fig.2 illustrates the dielectric barrier discharge reactor used in this study. Stainless steel mesh and aluminum plate were used as high voltage and GND electrodes, respectively. The high voltage electrode was 100mm x 100mm square made of SUS mesh (20mesh). Teflon sheet (2mm-thick) was set on the high voltage electrode as a dielectric barrier. The gap between the dielectric barrier and the GND electrode was adjusted by inserting the spacer. Uniform silent discharge was generated between the high voltage and the GND electrodes. A high voltage AC power supply (Kasuga-denki AGF-010) was used in this study. Fig.3 shows a photograph of the discharge. Filament-like electrical discharges were generated uniformly between the high voltage and the GND electrode. The peak to peak voltage, the frequency of the applied voltage and the input power were 24kVp-p, 34kHz and 230W, respectively. Typical waveform of the AC high voltage is shown in Fig.4. Ambient temperature during the experiment was room temperature about 27°C.

The sample was put on the GND electrode. Spores of *Bacillus Subtilis* were spotted on a polyethylene terephthalate (PET) films. The samples were prepared according to the following procedures indicated in Fig.5.

1. PET films 20mm x 20mm were sterilized by UV lump for 48hours.
2. *Bacillus Subtilis* spore suspension with concentration between \(10^7\) and \(10^8\) CFU/cm\(^3\) was prepared by diluting the original spore suspension.
3. The samples
   3-1) Dry sample: Spore suspension of 0.1ml was spread on the PET film and then dried at room temperature before use.
   3-2) Wet sample: Spore suspension of 0.1ml was spread on the PET film and then used without drying.

Application of the DBD plasma in surface sterilization of a belt conveyer was also examined. Fig.6 illustrates the system. The electrode consisted of a stainless steel rod inserted in a quartz glass tube (high voltage) and a metal base of the conveyer (GND). The glass tube, as a dielectric barrier, was 10mm outer diameter and 2mm thickness. The belt was also dielectric barrier. The spacing between the electrodes, or the length of the air gap was adjusted to be 2mm. This system reactor was driven by the AC power supply. The peak-to-peak voltage, frequency and input power were 24kVp-p, 27 kHz, and 230W, respectively.

The samples for the conveyer-type sterilization test were prepared as follows.

1. PET films 20mm x 20mm were sterilized by UV irradiation for 48hours.
2. Bacterium suspension was first prepared by incubating pork crushed liquid. The number of cell in the suspension was adjusted between \(10^6\) and \(10^8\) CFU/cm\(^3\) by diluting the original cell suspension.
3. Cell suspension of 0.1ml was spread on the PET film and then dried at room temperature before use.

CFU was determined using the film medium that selectively cultivated *E. coli*.
III. RESULT AND DISCUSSION

A. Effect of Sterilization using DBD Reactor

Time-lapse change of the effect of sterilization using the dry samples was measured. Fig. 7 shows the experimental result. We examined the effect of treatment time for 1, 2, 3 and 4 minute(s). Number of survivors decreased with increasing treatment time, and complete sterilization was achieved after 4 minutes of the treatment. D-value of the dry method was about 40 seconds. Speed of sterilization of this method was higher compared with conventional methods for Bacillus Subtilis spores. [1]

The experimental results of the dry and wet sterilization test suggest that the H₂O enhanced the effect of sterilization. There are many possible factors that might have enhanced the effect of sterilization. It is supposed that OH radicals were produced from H₂O, and sterilization was enhanced by OH radicals produced in the spores’ neighborhood. In addition, electrical breakdown of the cell membrane [6] is also another possible explanation of the sterilization enhancement by water addition. The current flowing through spores is increased by adding water. And water addition and heat might have induced the transition from spores to nursing cells, which are less resistant than spores.

Bacillus Subtilis spores after plasma treatment for 1 minute were visualized by phase contrast microscope (PCM). Fig. 9 and 10 show photograph of spores treated for 1 and 4 minute(s) by the dry method, respectively. Judging from the comparison with Fig. 1, no apparent difference of spore structure before and after plasma treatment was found in Fig. 9 and 10. Fig. 11 shows a photograph after the plasma treatment for 1 minute of the wet method, spherical spore of Bacillus Subtilis was almost destroyed and broken into fragments. This result suggests the wet method destroyed spores more apparently than that of the dry method. And mechanism of the dry sterilization method might be different from that of the wet method, because complete sterilization was achieved after 4 minutes of treatment by the dry method.

We found that the wet method was very effective but its application is somewhat limited. Therefore, we tried to modify the dry method to be more effective and widely-applicable one. In this experiment, the dry sample was used and 0.1 ml of pure water was supplemented just before the sterilization test. Plasma exposure was immediately done after the water addition.
Fig. 12 shows the experimental result using the biological indicator of *Bacillus Subtilis* for the dry sample with water addition, at 230W, 34kHz, and the treatment time of 10, 20, 30, 40, 50, 60 and 70 seconds, respectively. The number of the survivors decreased with increasing treatment time. Complete sterilization was achieved after 70 seconds of the treatment. And D-value was only about 12 seconds, which was much smaller than that of dry one. Effect of sterilization of this method was higher than that of the dry method, but it was slightly lower than that of the wet method. This may be possibly because the spores were coagulated and accumulated during preparation of the dry sample. Thus radicals may not diffuse inside the accumulated spores.

![Fig. 9 PCM Photograph of Bacillus Subtilis spores treated for 1 minute by the dry method](image1)

![Fig. 10 PCM Photograph of Bacillus Subtilis spores treated for 4 minutes by the dry method](image2)

Fig. 11 PCM Photograph of *Bacillus Subtilis* spores treated for 1 minute by the wet method

Fig. 12 Number of survivors of *Bacillus Subtilis* in dry method with water addition

![Graph showing number of survivors vs. treatment time](image3)

Fig. 13 shows the correlation between the input power and the effect of sterilization. The experimental conditions of sterilization were as follows. The input power were 100, 130, 160 and 230W. The frequency was 34kHz. The treatment time was 30 seconds. The wet sample was used in this experiment. It was found that survivability decreased exponentially with increasing the electric power. There are several mechanisms of plasma based sterilization: oxidation by radicals, breakdown of cell membrane due to high electric field, UV, etc. Detailed mechanism of the exponential correlation between the input power and the number of survivors was not clear yet.

The correlation between the effect of the sterilization and the discharge gap is summarized in Fig. 14, with 230W, 34.6 kHz and 30 seconds of the treatment time. The wet sample was used in this experiment. The discharge gap was varied as 1, 2 and 3 mm. The shorter gap (1 and 2 mm) resulted in high sterilization effect. But the 3 mm gap resulted in lower effect than the others. D-values of 1, 2 and 3 mm gap were 6.4 seconds, 5.6 seconds and 10.7 seconds, respectively. These are attributable to decreased density of the streamers with increasing gap distance, because less intense discharge was observed under 3 mm gap condition than 1 or 2 mm.
In order to examine the effect of the conductivity of the spore suspension, we conducted the DBD sterilization of the sample for the dry method with addition of salt water. Fig. 15 shows the effect of sterilization versus salt concentration at the input power of 230W, 34kHz and 30seconds of the treatment time in all the measurement. In this experiment, spore suspension on PET film was dried first then 0.1ml of salt water was added just before exposing to the plasma. Plasma exposure was immediately done after the addition of the salt water. These results show addition of 4g/L salt solution significantly enhanced the effect of sterilization. On the other hand, effect of sterilization using 8 and 10g/L salt water were lower than that using pure water. If the spores are covered with highly conductive liquid, they are electrically shielded and electrical membrane breakdown can be suppressed.

B. Conveyer type the Sterilization Reactor

We examined sterilization of a belt conveyer using DBD. The sample of E. coli, was used for this experiment, because E. coli is one of typical causes of food poisoning.

Fig. 16 shows the result of the experiment with the input power of 230W, 27 kHz. Dry sample was put on a belt conveyer and the speed of the conveyer was adjusted to 20mm/sec. Because the width of the discharge area was about 10mm, and the sample width was 20mm, the averaged plasma exposure time was 0.5sec/cycle. Number of the treatment was 1, 2, 3, 4cycle(s). Complete sterilization was achieved after 4cycles of treatment or 2seconds of treatment time. D-value was about 0.35seconds, which was short enough for practical use.

IV. CONCLUSIONS

Atmospheric pressure dielectric barrier discharge was used for sterilization, and following results were obtained.

1. In the most effective case for sterilization of Bacillus Subtilis spores, complete sterilization was achieved in 40seconds in the wet condition.
2. The effect of sterilization by DBD treatment became weaker with longer discharge gap, where weaker discharge plasma was generated.
3. Addition of 4g/L salt enhanced the effect of sterilization but 8g/L or more salt decreased the effect compared with the addition of pure water.
4. Complete sterilization of E. coli was achieved in 2seconds in the dry method using the conveyer sterilization system using DBD.

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REFERENCES


