Basic Study of Bacteria Inactivation at Low Discharge Voltage by Using Microplasmas

Kazuo Shimizu, *Member, IEEE*, Masahiro Yamada, Masaki Kanamori, and Marius Blajan

***Abstract*—Inactivation of microorganisms, such as *Escherichia coli*, by exposure to a microplasma is experimentally investigated. A microplasma is an atmospheric-pressure nonthermal plasma. Microplasmas, which generate high-intensity electric fields, can be formed using relatively low discharge voltages (0.7–1.1 kV) across small discharge gaps (0–100 μm). The key benefits of the practical application of exposure to a microplasma are as follows: 1) the low discharge voltage and 2) the simple apparatus because a vacuum enclosure is not required. Hence, the apparatus for generating a microplasma could be relatively small and inexpensive and could be integrated into a portable device. The ozone generated by a microplasma at a low power level was measured, although the specific power density of the microplasma was larger than that of large-scale conventional plasmas. The emission spectra of the microplasma discharge in N_2 was measured: 1) to confirm the UV light emission and 2) to identify the active chemical species generated by the microplasma discharge. The emission spectra was also measured with the presence of water droplets. The UV light from the microplasma discharge showed excited nitrogen molecules and OH radicals. In this paper, two cultures of bacteria, i.e., gram-negative *Escherichia coli* HB101 and gram-positive *Bacillus subtilis* JCB 20036 were the target microorganisms to be inactivated. In the experiments reported here, the number of bacteria decreased after microplasma treatment. The inactivation rate increases as the discharge voltage increases. *Escherichia coli* is completely inactivated when air is used as carrier gas at a plasma discharge voltage of 1.05 kV. Using nitrogen as carrier gas, the highest inactivation rate is 77% at a discharge voltage of 1.15 kV. In addition, *Bacillus subtilis* is inactivated with a rate of 97% at 1.07 kV with air as carrier gas. Using nitrogen as carrier gas and a discharge voltage of 1 kV results in an inactivation rate of 70% of bacteria. The inactivation of microorganisms by microplasma may be due to several factors either individually or in combination of the following: 1) the excited molecules and ions; 2) ozone; 3) high electrical fields; and 4) UV light. The effect of active species such as OH radicals may also be important since all the bacteria were carried within a small water droplet in between the electrodes.**

***Index Terms*—*Bacillus subtilis*, electric fields, *Escherichia coli*, inactivation, microplasma, ozone.**

I. Introduction

TRADITIONALLY, heat sterilization [1], irradiative sterilization [2], and chemical treatment [3] are used for sterilization. If these methods were applied to food, for example, concerns would include the deterioration of flavor by the heating and contamination by residual chemicals. Therefore, an effective sterilization method is needed that is safe and does not affect the foods' flavor. Recently, sterilization methods using various kinds of discharges and plasmas have been investigated [4]–[16]. The plasma can be applied to heat-sensitive materials without any thermal damage, and it can also be applied to human skin without any sensation of pain.

Nonthermal plasmas generated by dielectric-barrier discharges at atmospheric pressure have several different mechanisms occurring simultaneously that may be germicidal: UV radiation which may reach into the vacuum UV region, and streamers which contain high-energy electrons, which generate short-lived chemically reactive radicals by electron impact dissociation of molecular gases [11]. Nonthermal plasma with H_2O addition shows higher sterilization rates than those of dry processes [12]. Atmospheric-pressure glow discharge with helium results in a higher efficiency of *Bacillus subtilis* spore inactivation than that of a helium–oxygen plasma plume [13]. Nonthermal plasmas for biodecontamination, chemical decontamination, plasma decontamination in medicine, and electric discharges for plasma decontamination have been studied and reported by various groups [14].

In this paper, the inactivation by microplasma was investigated, in which the discharge voltage and power were lower in comparison with conventional plasma sterilization. Two cultures of bacteria were used to carry out the inactivation experiments: gram-negative *Escherichia coli* HB101 and gram-positive *Bacillus subtilis* JCB 20036 cultures, respectively. In order to investigate the impact of different radical species formed in the microplasmas, the experiments were performed using two different carrier gases: air and nitrogen.

II. About Microplasma

Fig. 1 shows a schematic image of the electrodes used in the experiments. A ferroelectric material covered the surface

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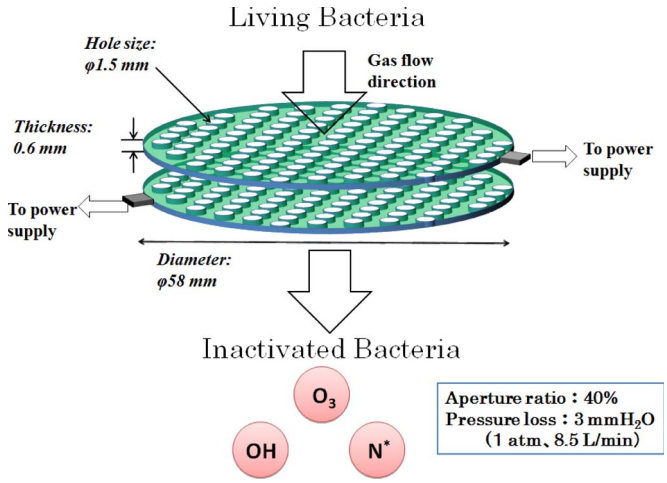


Fig. 1. Schematic image of microplasma electrodes. The pressure loss between the electrodes is very small.

TABLE I
MINIMUM SPARKING VOLTAGE AND DISCHARGE GAP FOR VARIOUS GASES

Gas	Minimum sparking voltage [V]	Discharge gap [μm]
Air	330	7.5
Oxygen	450	9.2
Nitrogen	275	9.9

of the stainless-steel electrodes [17], [18]. The diameters of the electrodes are ∅58 mm, and the thickness of each electrode is 0.6 mm.

The thickness of the dielectric barrier covering the base metal is about 100 μm. The electrodes have holes (∅1.5 mm), and their aperture ratio is about 40%. The pressure loss is relatively low (about 3-mm H_2O at a gas-flow rate of 8.5 L/min). By applying an alternating voltage to the electrodes, streamers are formed between the electrodes.

In the experiments reported here, the discharge gap between the electrodes is set to 10 μm in order to minimize the discharge voltage at atmospheric pressure based on Paschen's law.

Table I shows the sparking voltages and the discharge gaps for air, nitrogen, and oxygen.

Fig. 2 shows a schematic image of the active species generated by the microplasma between the electrodes and the exposure of the living bacteria to the microplasma causing inactivation. A suspension of living bacteria in water was sprayed perpendicular to the electrode. The living bacteria were carried through the holes by the carrier gas. Due to the small discharge gap between electrodes (0–10 μm) and the direction of the gas flow, few of the bacteria enter the discharge gap between electrodes, and most pass through the holes. The few bacteria that enter the narrow zone between the electrodes attach to the surface of the electrodes and are lost from the flow of the carrier gas. The bacteria and their colonies that were counted on the nutrient medium were exclusively those bacteria which passed the holes.

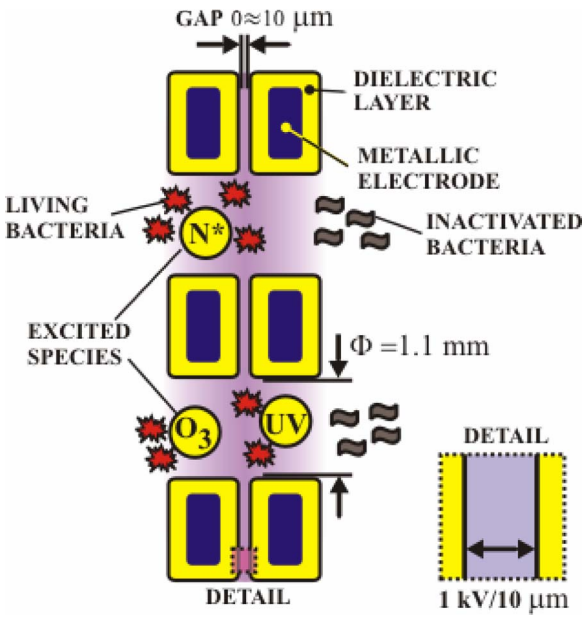


Fig. 2. Schematic image of the inactivation processes of microplasma between the electrodes.

A microplasma is a dielectric-barrier discharge characterized by a large number of short-lived microdischarges. Each microdischarge has an almost cylindrical plasma channel of about 100-μm radius [19].

Thus, the electrodes have a plasma area of 0.314 mm^2 located around each hole. The area in which the plasma does not occur is 0.64 mm^2 located in the center of each holes. In this region, the excited nitrogen molecules, nitrogen ions, ozone, and the UV light interact with the bacteria resulting in inactivation, as shown in Fig. 2 and Section VII [20], [21]. It is reported that each bacterium can be attacked and degraded by the ozone and the active species generated by the plasma [11].

The dielectric barrier with a thickness of 100 μm has an assumed specific dielectric constant of $\varepsilon_{r1} = 10^4$. The electric displacement field inside the capacitor formed by the two dielectric layers and the air gap is constant; in the air gap, the electric field is $E_2 = 10^4 E_1$, where E_1 is the electric field in the dielectric layers. Thus, the discharge gap is set to less than 10 μm to generate a high electrical field ($E_2 = 10^7$–10^8 V/m) and assure the formation of nonthermal plasmas at a discharge voltage of around 1 kV.

In addition, the high electron temperatures ensure the production of chemically active species. In general, bacteria can be sterilized with an electric field of about 10^4–10^5 V/m [22].

III. EXPERIMENTAL SETUP

The experimental setup is shown in Fig. 3. *Escherichia coli* HB101 (gram-negative bacteria) and *Bacillus subtilis* JCB 20036 (gram-positive bacteria) cultures were used for our inactivation studies. Air or nitrogen were supplied through the flow meter and mixed with a 5-mL culture suspension in the nebulizer. Cultured suspensions of *Escherichia coli* and *Bacillus subtilis* containing 10^7 colony-forming units /mL were prepared.

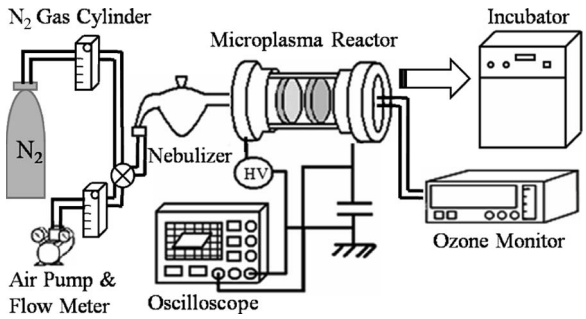


Fig. 3. Experimental setup for inactivation of microorganisms using a microplasma reactor.

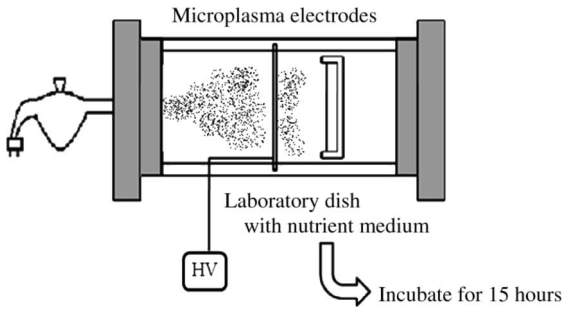


Fig. 4. Detailed assembly of the microplasma reactor.

The cultured suspensions were introduced into the microplasma reactor and sprayed through the electrode against a plate with nutrient agar medium at a gas-flow rate of 7 L/min. The desired gas-flow rate was adjusted by a pump or by the inner pressure of the nitrogen gas cylinder.

A digital oscilloscope (Tektronix, TDS 2014), a high-voltage probe (Tektronix, P6051), and an ac transformer (Tektronix, P6021) were used to measure the discharge voltage and corresponding discharge current.

The ozone concentrations were measured with an Ozone Monitor (Ebara Jitsugyo, EG-2001B), and the bacterial cultures treated by microplasma were incubated with agar medium in plates.

A high-voltage power supply (LECIP, M-1H) was used to apply a high-frequency ac voltage (25 kHz, 1 kV) to the microplasma electrodes.

Fig. 4 shows the details of the microplasma reactor. The inside diameter, the length, and the volume of the reactor were 48 mm, 180 mm, and about 330×10^3 mm^3, respectively. The sprayed suspensions were passed through the electrodes, and they accumulated on the culture plates. Experiments with and without microplasma were performed under equal conditions for the control.

After the plasma treatment of the bacteria and their collection on culture media, the culture plates were incubated in an incubator (Tokyo Rikakikai, LTI–700E) at 37 °C for 15 h for *Escherichia coli*, and 30 °C for 18 h for *Bacillus subtilis*.

The inactivation rates were calculated from the averages (experiments were carried out in triplicate) by comparing the number of colonies with and without microplasma treatment. Some bacteria in water droplets were lost to the surface of the electrodes during the microplasma treatment by electrostatic precipitation.

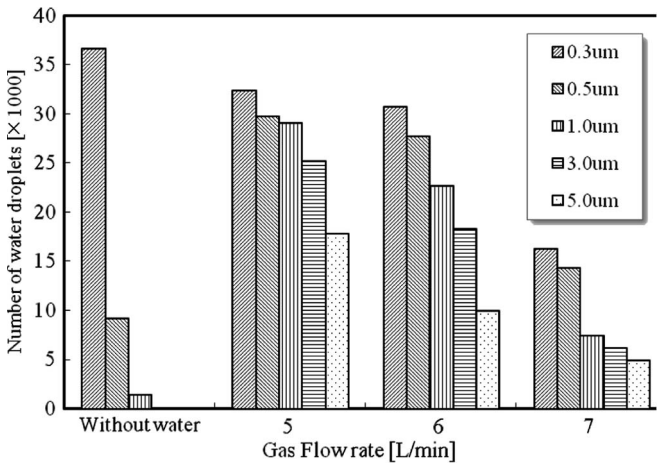


Fig. 5. Size distribution of water particles generated inside the nebulizer at gas-flow rates of 5, 6, and 7 L/min.

IV. Characteristics of the Particles by the Nebulizer

The diameters of the water particles formed by the nebulizer were measured by a laser particle counter (Kanomax, 3886).

The gas-flow rate was restricted to the range of 5–7 L/min because the pressure loss of the nebulizer was high. With no water added to the nebulizer, no droplets were formed. As shown in Fig. 5, our particle counter observed particles with diameters of only 0.3 and 0.5 μm. These particles could be the dust in the air. With water added to the nebulizer, water droplets with diameters ranging from 0.5 to 5 μm were generated at the applied gas-flow rates of 5, 6, and 7 L/min. Smaller water droplets did not coalesce to form larger droplets because the number of 0.5-μm droplets exceeds the number of 1.0-μm droplets. Since the sizes of colon bacilli *Escherichia coli* HB101 and *Bacillus subtilis* JCB 20036 are about 0.5 to 2.5 μm, they are contained within the water droplets generated by the nebulizer.

V. Electrical Characteristics

In order to investigate the effect of spraying the microplasma electrodes with water, the discharge current was measured while spraying distilled water.

Fig. 6 shows an example of discharge waveforms. Current spikes [Fig. 6(b)] were observed at the steepest slopes of the discharge voltage [Fig. 6(a)]. This is a typical waveform of a dielectric-barrier discharge [19].

Fig. 7 shows the stability of the discharge current over time. Initially, the discharge current decreased slightly with time, and after 5 min, the discharge current was stable. It is possible that a portion of the electrode surface was covered by water, which prevented the electrical discharge.

Such a phenomenon would result in the observed decrease in the discharge current.

Fig. 8 shows thermal camera pictures taken after 5 min of discharge. The temperature of the electrodes was almost the

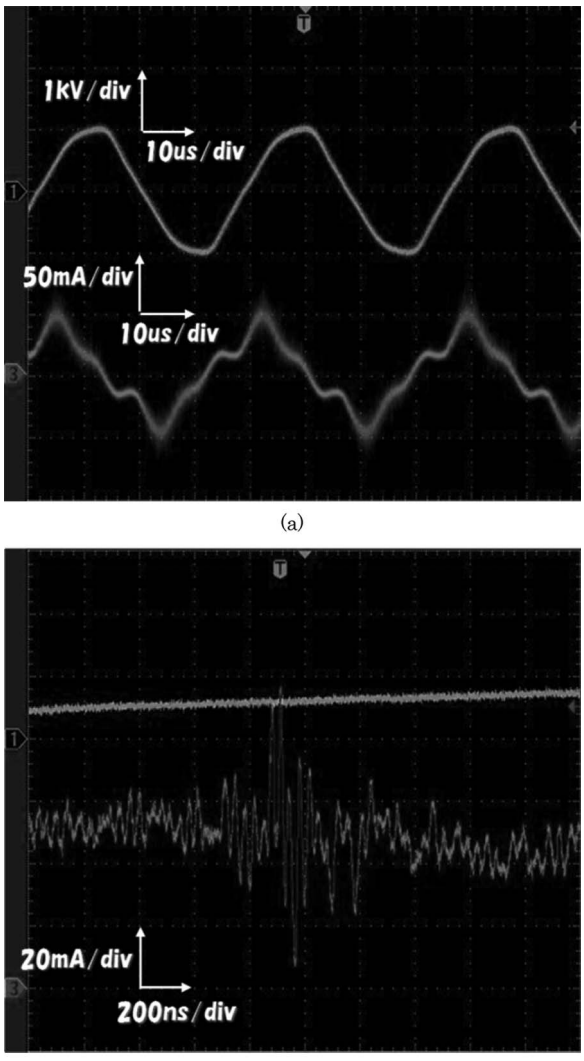


Fig. 6. Examples of discharge waveforms at 1 kV using air as the carrier gas. (a) Discharge voltage. (b) Discharge current.

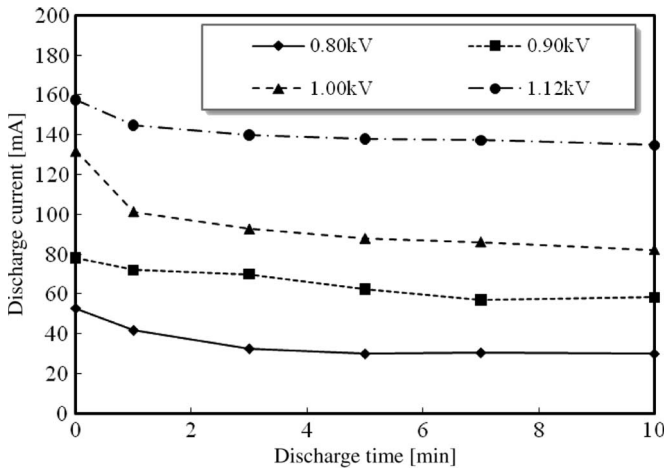


Fig. 7. Change of discharge current after discharge start with water spray.

same (45 °C) for both with and without sprayed water droplets. After 5 min, the temperature of the electrodes and the discharge current were stabilized even with water droplets.

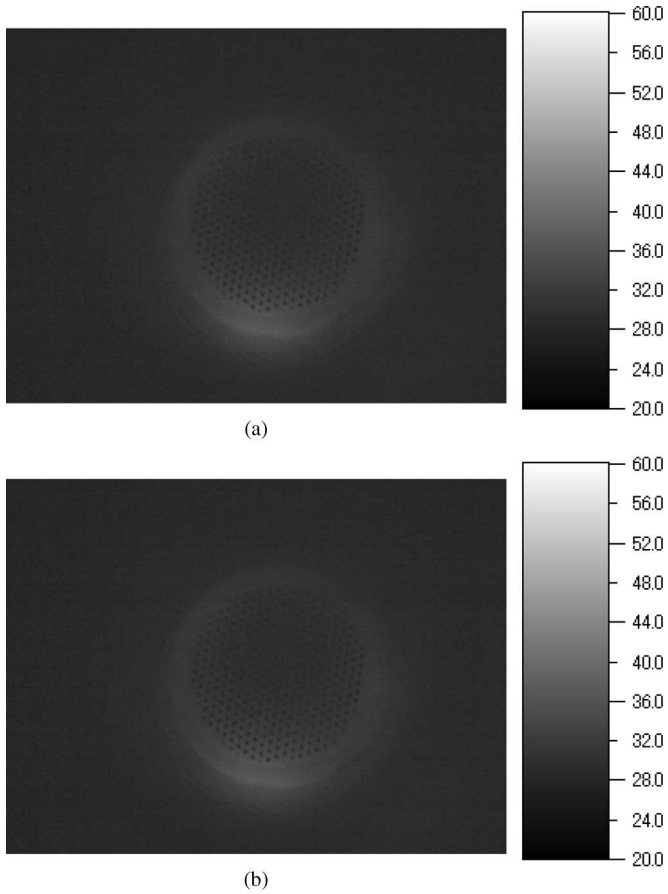


Fig. 8. Images of the thermal distribution of electrodes with and without distilled water droplets at a gas-flow rate of 7 L/min. (a) Thermal distribution of electrodes without water droplets. (b) Thermal distribution of electrodes with water droplets.

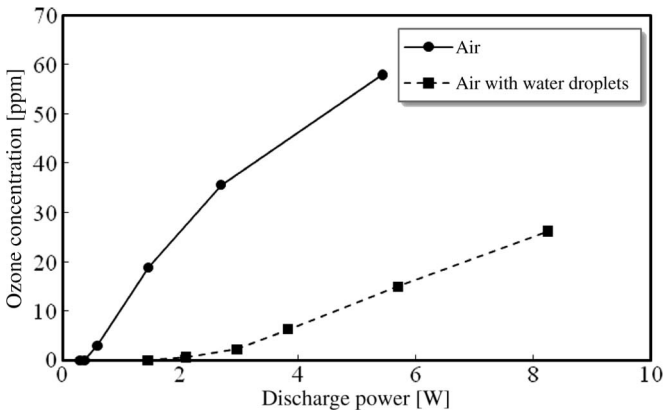


Fig. 9. Ozone concentration increases with discharge power both with and without water droplets at a gas-flow rate of 5 L/min.

Water droplets sprayed by the nebulizer are considered to have a minor influence on the discharge phenomena.

VI. Ozone Generation

The concentrations of ozone generated by the microplasma increased with discharge power at a gas-flow rate of 5 L/min, as shown in Fig. 9. Ozone concentrations increased almost linearly with discharge power with no water droplets present.

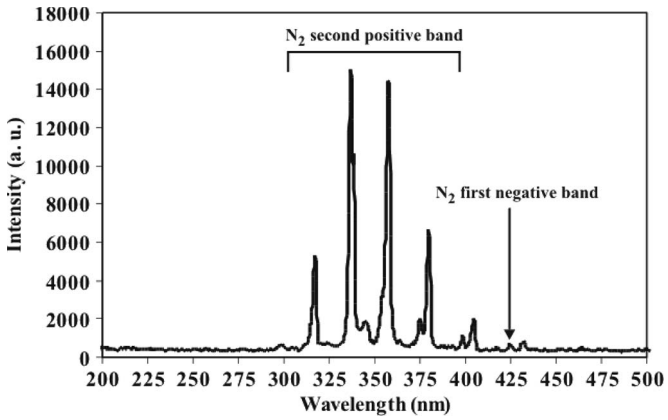


Fig. 10. Emission spectrum of the microplasma discharge in N_2. Gas-flow rate was set at 5 L/min.

With water droplets present, the flowing gas was cooled down and condensed with iced water before measuring the ozone concentration for accurate measurements. The ozone concentrations were lower than for the same discharge power without water droplets due to the reaction by water [19]. Optical measurements discussed in the next section did not show any ozone peaks in the microplasma since the ozone is generated in the afterglow region of the discharge section.

Ozone is generated by a three-body slow reaction of free radical atomic oxygen and the ozone also dissociated by electron and atomic oxygen [23], [24].

VII. UV Light Emission by Microplasma

UV light emissions from microplasma were observed to confirm the effect of UV light on inactivation process of bacteria [20]. The emission spectra were measured by an intensified charge-coupled device (ICCD) camera (Ryoushi-giken, SMCP–ICCD 1024 HAM-NDS/UEmV), a spectrometer (Ryoushi-giken, VIS 351), and by a photomultiplier tube (Hamamatsu Photonics, R3896). A pulse generator (Tektronix, AFG 3021B) was used to trigger the ICCD camera and the Marx generator consisting of semiconductor switches. A Marx generator with four-stage MOSFET switches was used as the high-voltage supply for the microplasma electrodes. The spectrum was observed at -1.4 kV with a pulsewidth of 500 ns and a frequency of 1 kHz. The gas-flow rate of dry nitrogen was set at 5 L/min. Data obtained from the ICCD camera were transferred to a computer for analysis.

The emission spectrum of the microplasma discharge in N_2 shown in Fig. 10 shows peaks of N_2 second positive band system (N_2 SPS) and N_2 first negative band system (N_2 FNS). The spectrum indicates the generation of active molecular nitrogen species in the microplasma discharge [25].

The elementary processes (1) and (2) describe the radiation kinetics for the SPS of nitrogen with a wavelength of 337.1 nm and at atmospheric pressure. [26].

The excitation of nitrogen molecules in the ground state by direct electron impact is described by

$$e + \mathrm{N_2}\left(X^1\Sigma_g^+\right)_{v=0} \rightarrow \mathrm{N_2}(\mathrm{C}^3\pi_u)_{v'=0} + e \quad (\Delta E = 11\ \mathrm{eV}). \tag{1}$$

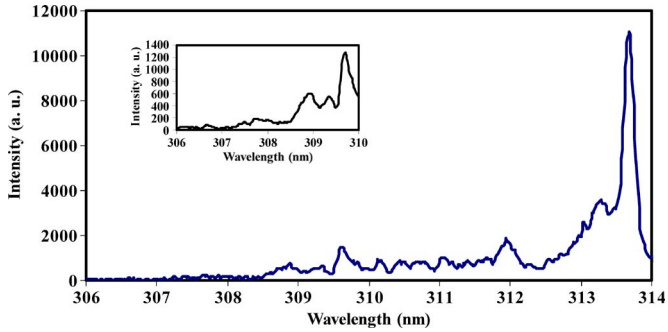


Fig. 11. Emission spectrum of OH in microplasma discharge with nitrogen and water droplets using a nebulizer. Gas-flow rate was set at 5 L/min. Emission spectra was observed at -1.2 kV. The other conditions were the same as described in Fig. 10.

The spontaneous radiation of nitrogen in the excited state is described by

$$\mathrm{N_2}(\mathrm{C}^3\pi_u)_{v'=0} \rightarrow \mathrm{N_2}(\mathrm{B}^3\pi_g)_{v''=0} + hv \quad \left(\tau_0^C = 40\ \mathrm{ns}\right). \tag{2}$$

Water droplets from the nebulizer were entrained in the nitrogen gas that entered the chamber to confirm another active species in the microplasma discharge. Fig. 11 shows the emission spectrum of nitrogen with entrained water droplets. The gas-flow rate was set at 5 L/min. The UV light emission confirmed active species such as OH radicals around 307 to 309 nm, as shown in Fig. 11 [25], [27]. UV light emissions from 316 nm and higher wavelengths were also observed, which affect the inactivation process of bacteria [28], [29]. The combination of UV light and active species could have contributed to the inactivation of bacteria.

VIII. Inactivation by Microplasma

From these experiments, the basic characteristics of microplasma, such as discharge current, discharge power, and ozone generation in air, were obtained. Inactivation of *Escherichia coli* and *Bacillus subtilis* were experimentally investigated at a gas-flow rate of 7 L/min by using microplasma electrodes. Ambient air and nitrogen were used to compare the effect of the oxidization effect of ozone and to confirm the effect of high electric field and UV radiation from microplasma.

Figs. 12–15 show photographs of the culture plates of treated *Escherichia coli* and *Bacillus subtilis* incubated for 15 h after treatment with a microplasma using air or nitrogen as the carrier gas. A decrease of the number of colonies was observed when the discharge voltage increased in both air plasma and nitrogen plasma. The inactivation process for bacteria may occur between the electrodes or in the space near the electrodes after passing through the holes in the electrodes.

Because of some limitations of the experimental setup, some colonies gathered at the lower edge of the nutrient medium and were difficult to count. Therefore, these uncountable colonies were excluded, and colonies near the center of the dish were used for comparison.

Fig. 16 shows the inactivation rates of *Escherichia coli* versus discharge voltages for the carrier gases, air and nitrogen,

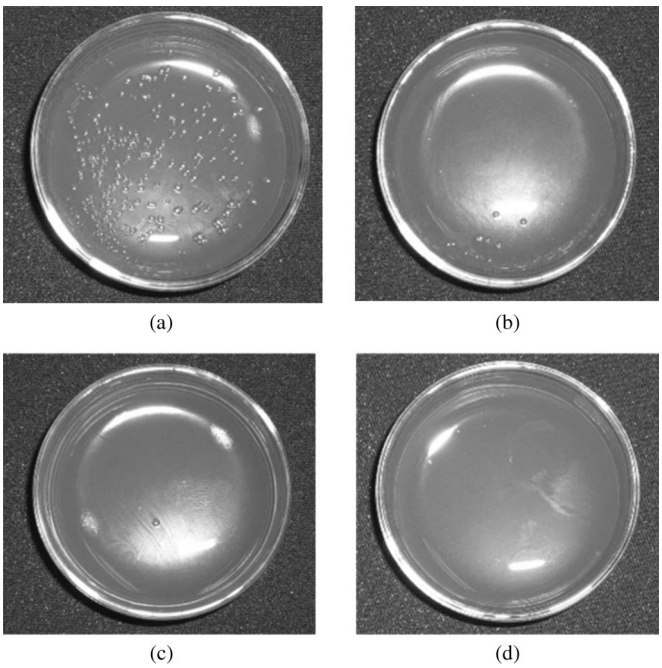


Fig. 12. Images of the *Escherichia coli* treated samples by microplasma after 15 h of incubation. Carrier gas is air at a gas-flow rate of 7 L/min. After the treatment by microplasma at (d) the discharge voltage of 1.08 kV, no colonies are observed. (a) Without discharge. (b) Discharge voltage of 0.90 kV. (c) Discharge voltage of 1.02 kV. (d) Discharge voltage of 1.08 kV.

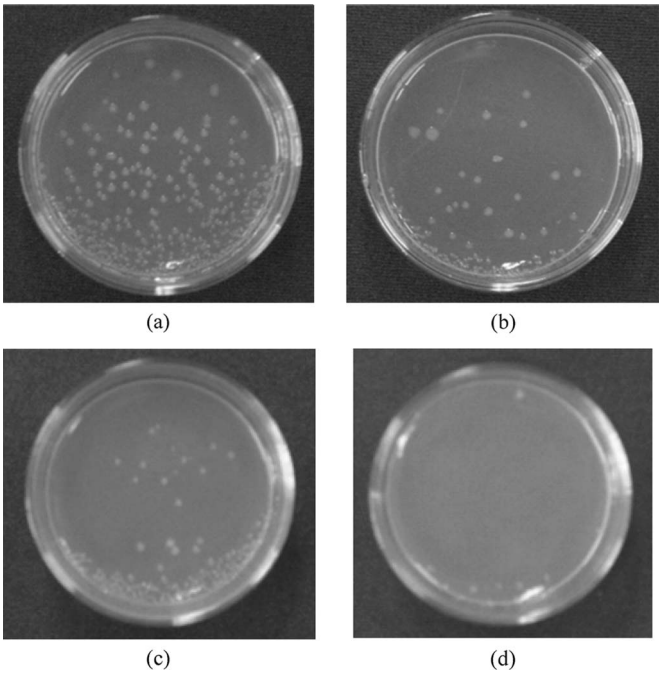


Fig. 13. Images of the *Escherichia coli* treated samples by microplasma after 15 h of incubation. Carrier gas is nitrogen at a gas-flow rate of 7 L/min. Few colonies still exist after the treatment by microplasma at (d) the discharge voltage of 1.15 kV. (a) Without discharge. (b) Discharge voltage of 0.76 kV. (c) Discharge voltage of 0.90 kV. (d) Discharge voltage of 1.15 kV.

respectively. When nitrogen was the carrier gas, the inactivation rate had the maximum value of 77% corresponding to a discharge voltage of 1.15 kV. Ozone was not formed during the discharge in the presence of nitrogen, and the inactivation of *Escherichia coli* could be considered to be due to the effects

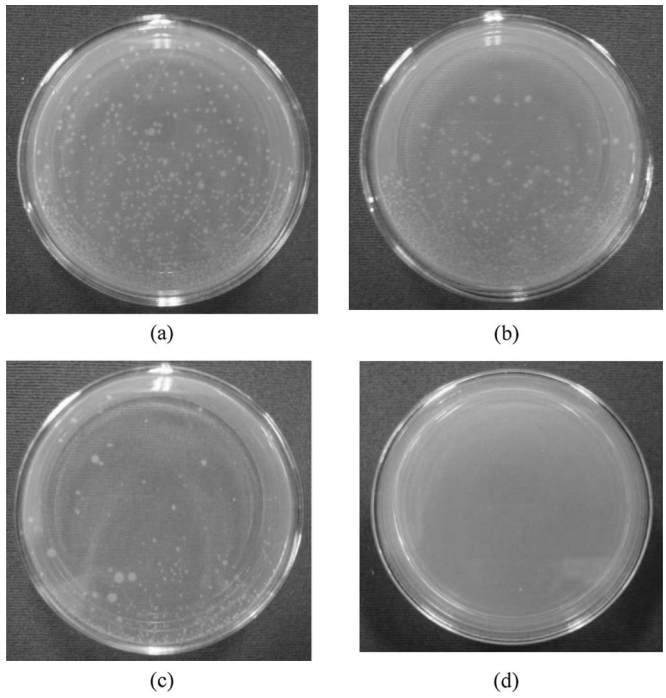


Fig. 14. Images of the *Bacillus subtilis* treated samples by microplasma after 15 h of incubation. Carrier gas is air at a gas-flow rate of 7 L/min. Few colonies still exist after the treatment by microplasma at (d) the discharge voltage of 1.07 kV. (a) Without discharge. (b) Discharge voltage of 0.77 kV. (c) Discharge voltage of 0.85 kV. (d) Discharge voltage of 1.07 kV.

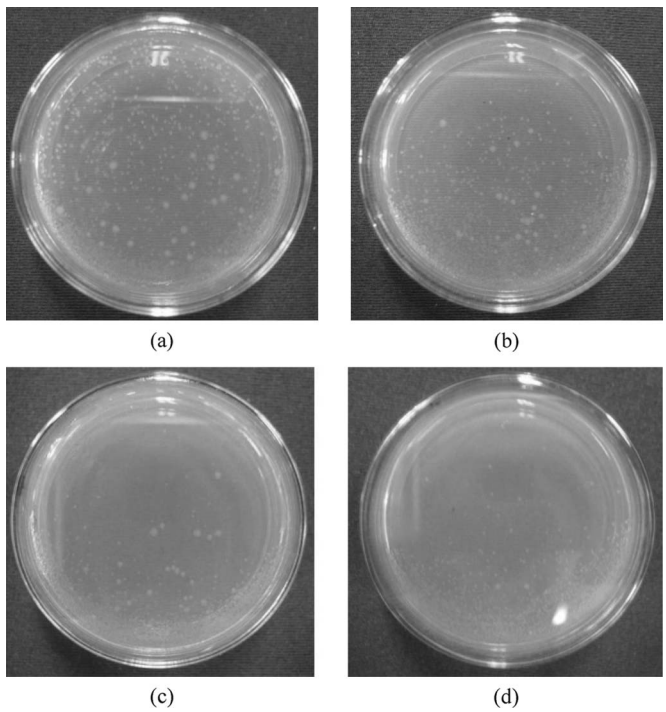


Fig. 15. Images of the *Bacillus subtilis* treated samples by microplasma after 15 h of incubation. Carrier gas is nitrogen at a gas-flow rate of 7 L/min. Few colonies still exist after the treatment by microplasma at (d) the discharge voltage of 0.96 kV. (a) Without discharge. (b) Discharge voltage of 0.67 kV. (c) Discharge voltage is 0.81 kV. (d) Discharge voltage of 0.96 kV.

of high electric field, excited nitrogen ions, active species such as OH, and UV radiation by microplasma, as shown in Figs. 10 and 11.

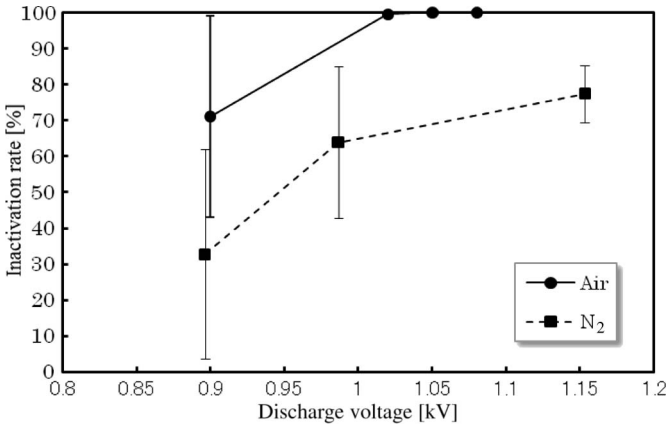


Fig. 16. Comparison of inactivation rate of *Escherichia coli* with air and nitrogen plasma.

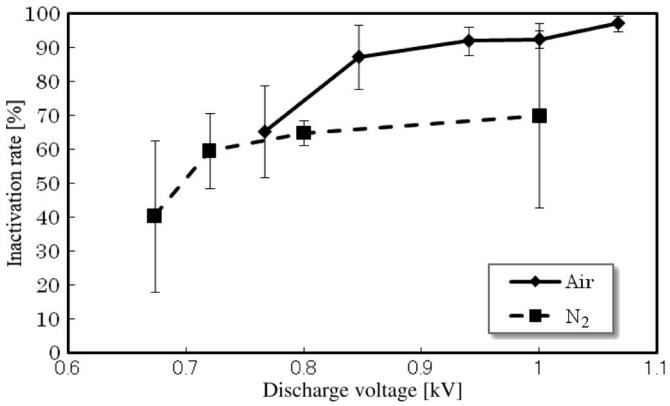
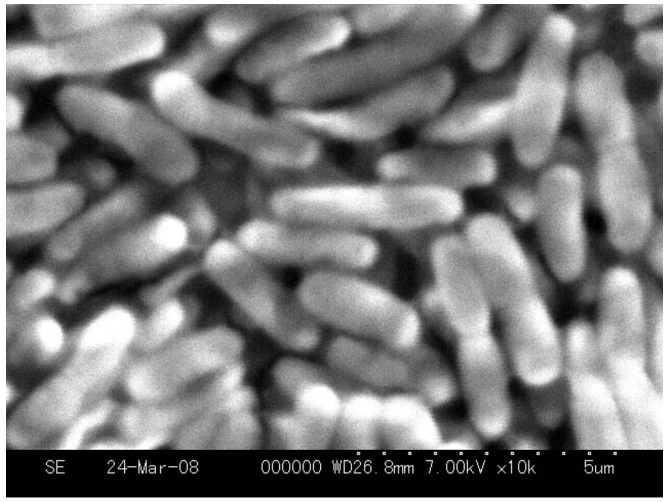
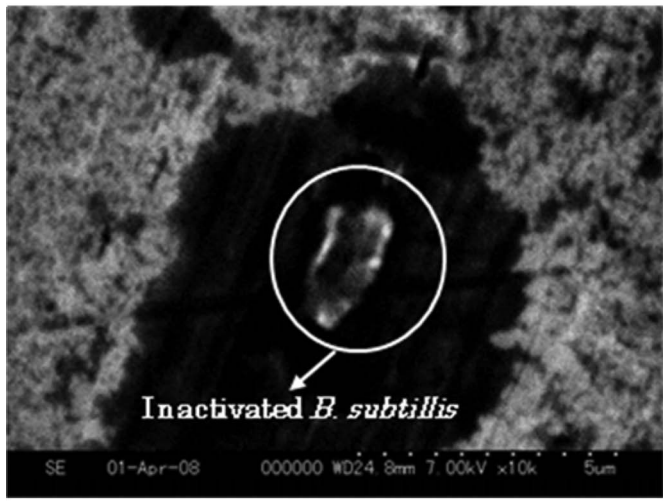


Fig. 17. Comparison of inactivation rate of *Escherichia coli* with air and nitrogen plasma.



(a)



(b)

Fig. 18. Images of *Bacillus subtilis* by SEM. (Gas-flow rate 7 L/min, discharge voltage = 1 kV, N_2: carrier gas). (a) Living *Bacillus subtilis* ($\times$10 000 photograph taken by SEM). (b) Sterilized *Bacillus subtilis* by nitrogen plasma ($\times$10 000 photograph taken by SEM).

In the case of the air as carrier gas, the inactivation rate was 100% at 1.05 kV. The higher inactivation rates obtained after air-plasma treatment were due to the combined effects of ozone, other active species, high electric field, and UV radiation by microplasma. In particular, the effect of UV on deoxyribonucleic acid (DNA) and the ozone on cell wall are well known [30], [31].

Fig. 17 shows the inactivation rate of *Bacillus subtilis* versus discharge voltages for the carrier gases, air and nitrogen, respectively.

In the case of air as carrier gas, the inactivation rate had a maximum value of 97% at 1.07 kV, and when nitrogen was the carrier gas, the maximum inactivation rate was 70% at 1 kV. These results are similar to the results of the inactivation of *Escherichia coli* due to the effects of ozone and active species existing in air plasma.

More effective results were obtained for the inactivation of *Escherichia coli* (gram-negative bacteria). It is possible that the lower inactivation of *Bacillus subtilis* (gram-positive bacteria) is caused by its relatively impermeable cell walls, which have a thickness in the range of 22 to 25 nm. The cell wall of gram-positive bacteria is composed of peptidoglycan and secondary polymers. Gram-negative bacteria have thin peptidoglycan layers (2–3 nm) plus an overlying lipid-protein bilayer (7–8 nm) known as the outer membrane [31]–[33]. Although the results show that the inactivation rates for gram-positive and gram-negative bacteria are different, detailed studies should be done to explore the mechanisms responsible for the different survival rates. If the mentioned differences in the structure of the cell walls or other processes are responsible for the different inactivation rates, they need to be clarified in future studies.

Fig. 18 shows the images of *Bacillus subtilis* before and after inactivation by nitrogen plasma. The images were taken by a scanning electron microscope (SEM, Hitachi, S-3000 N). An aluminum foil was disinfected and cleaned with alcohol to collect and transfer the incubated bacteria on the medium surface, as shown in Fig. 18(a).

The image of Fig. 18(b) shows the treated bacteria, *Bacillus subtilis* by microplasma (gas-flow rate is 7 L/min, discharge voltage = 1 kV, N_2 as carrier gas) on the medium surface which was also collected by using the aluminum foil. It shows how the cell wall of the bacteria or its shape was affected

by the microplasma discharge. In particular, active radical species has etching effect to break their cell wall, and UV affects the DNA directly to cut their structure [31].

IX. Conclusion

In this study, the inactivation of *Escherichia coli* and *Bacillus subtilis* using microplasma electrodes was experimentally investigated. The following conclusions are drawn from the series of experiments reported in this paper.

1) Other researchers report [22] that general bacteria can be inactivated with an electric field of about 10^4–10^5 V/m. Thus high-intensity electric fields of 10^7–10^8 V/m, which can be obtained by microplasma discharge, can be an inactivation mechanism for both *Escherichia coli* and *Bacillus subtilis*. A partial inactivation can be achieved using microplasma even without ozone in nitrogen as carrier gas.
2) A stable discharge was obtained using an applied voltage in the range 0.8–1.1 kV. A stable discharge was obtained even with some water present on the electrodes from droplets formed using a nebulizer that were sprayed against the electrodes.
3) The inactivation effect was confirmed at a relatively low discharge voltage of 0.9 kV, using either air or nitrogen as carrier gas.
4) The inactivation rate of both bacteria, *Escherichia coli* and *Bacillus subtilis*, increased as the discharge voltage increased with air and nitrogen used as carrier gases.
5) For *Escherichia coli*, a 100% inactivation was obtained at 1.05 kV with the application of air as carrier gas. Under otherwise equal conditions but with nitrogen as carrier gas, the inactivation rate decreased to 77% at 1.15 kV. The inactivation of bacteria could be considered as the combination effects of high electric fields, UV radiation, ozone, and active radicals formed inside the microplasma.
6) For *Bacillus subtilis*, the inactivation rate was 97% at 1.07 kV and with air as carrier gas. When the carrier gas was nitrogen, an inactivation rate of 70% was obtained at 1 kV. The higher rates obtained with air as carrier gas were due to the presence of the ozone and oxidative radicals.
7) The higher inactivation rates obtained for *Escherichia coli* compared with *Bacillus subtilis* were possibly due to the different characteristics of the cell walls of gram-positive and gram-negative bacteria, which have different thicknesses and chemical compositions.
8) Emission spectrometry was carried out to confirm the UV light and active species generated from microplasma. UV light ranging from 316 nm to a higher wavelength was observed which was mainly emitted from N_2 only in nitrogen gas.

Emission by OH radicals ranging from 306 to 309 nm was also observed, when nitrogen and water droplets flowed through the microplasma electrode.

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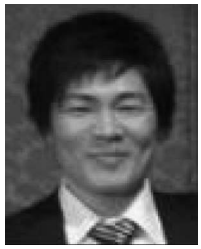

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