Generation and transport mechanisms of chemical species by a post-discharge flow for inactivation of bacteria

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Abstract. A post-discharge flow that is formed downstream of a microwave argon plasma in atmospheric air was investigated to clarify the generation and transport of chemical species, which are considered to result in the inactivation of bacteria. The flow, which is characterized by ultra-weak emission, can be visualized using an optical analysis system. This visualized jet-like flow forms downstream of the nozzle exit, and then, as the gas temperature is 877 K at the center of the nozzle exit, the main flow travels upstream around the quartz tube due to buoyancy, the reason being that the temperature decreases to room temperature at 30 mm downstream. It was clarified that excited argon atoms, molecular nitrogen (N_2 second positive system) and OH radicals were generated in the post-discharge flow, subsequent to which NO_2 and ions with a number density of 10^6 counts cm^{-3} were transported downstream below the main flow. These results imply that most of the heat and chemical species were transported by convective transport of the main flow, but that a small amount of chemically active species and ions might have been transported further downstream by diffusive transport, these species being considered to result in an inactivation effect on bacteria.

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1. Introduction

Pandemic infections of bird flu and severe acute respiratory syndrome (SARS) as well as nosocomial infection of Methicillin-resistant *Staphylococcus aureus* (MRSA) and other pathogenic bacteria greatly disrupt our daily life and constitute significant social problems. Thus, the establishment of safe and easy-to-handle disinfection and sterilization technologies is essential to decrease the risk of infection. Conventional sterilization methods in the medical field include high-pressure vapor sterilization, ethylene oxide gas sterilization and gamma-ray radiation sterilization. Each of these methods has disadvantages, for instance, long aeration time to remove excess gas or by-products, the need for large facilities and so on.

To solve these problems, sterilization methods using low-temperature plasma flow at atmospheric pressure have been developed [1]–[4]. There are several methods to produce such plasma, including dc discharge, RF discharge, dielectric barrier discharge (DBD) and pulsed corona discharge. Sato *et al* have already reported the sterilization efficacy and mechanism in a tube, using DBD [5, 6]. These methods, however, require that O and OH radicals are generated by using oxygen, air, hydrogen peroxide and so on, which results in the production of toxic gases such as ozone and nitrogen oxides. To apply plasma sterilization in open space, such as in houses, hospitals and day-care centers, it is necessary to use gases that do not harm the human body. Therefore, it is essential that the generation of toxic by-products is decreased to a safe level by the use of a different system. Shimizu *et al* have applied a microwave argon plasma flow to treat dermatoses [7], Sladek and Stoffels have developed a plasma needle using helium gas for plasma treatment [8], and Weltmann *et al* have also developed atmospheric pressure plasma jets (APPJs) for antimicrobial treatment [9]. Deng *et al* have sterilized bacteria using atmospheric-pressure glow discharges with helium gas [10]. Furthermore, Herrmann *et al* have clarified that chemically active species are effective for the sterilization of bacteria [11]. Moisan *et al* have also clarified the effectiveness of chemically active species for sterilization [12]. Laroussi and Leipold have reported the effects of UV, heat and reactive species on inactivation of bacteria [13], and Kim *et al* have applied species generated by using atmospheric air plasma to kill cancer cells [14].

In our previous studies, as an alternative to the aforementioned plasma devices, we used a new type of atmospheric low-temperature plasma source, which utilizes a coaxial plasma source
rather than a transmission type of microwave plasma source with a conventional waveguide, although a coaxial plasma source at low pressure has been previously reported [15]. This plasma source is advantageous at low temperature and is characterized by portability, simple configuration and operation at atmospheric pressure and low power. The post-discharge flow is advantageous in that it is capable of transporting chemical species by a flow and of suppressing the generation of toxic by-products by the use of noble gases such as helium and argon. However, the mechanism of radical generation and transportation in the post-discharge flow for sterilization has not been sufficiently clarified, although several studies have examined it. The authors have already reported that the flow can sterilize the spores of *Geobacillus stearothermophilus* (ATCC 7953) in bioindicator ATTEST No.1291, available from 3M Co. In that report, *G. stearothermophilus* was sterilized by at least $10^{-5}$ using pure argon gas under an exposure temperature of 383 K and an exposure time of 20 min [16]. This exposure temperature and time are relatively low compared with those of dry sterilization.

Clarification of sterilization mechanisms, such as damage to the cell wall and membrane, inactivation of enzymes, decomposition of RNA and ribosomes, and damage to chromosomes and DNA, is very important for further improvement of the atmospheric plasma sterilization system. In the above-cited study, the authors focused on the effect of post-discharge flow on the cell and cytoplasmic membranes of *Escherichia coli*. When the quartz glass was set above the test sample, the number of surviving cells of *E. coli* decreased by more than three orders of magnitude, although there was no marked decrease in the case of a UV-cut filter. The number of surviving cells of *E. coli* decreased by more than three orders of magnitude when *E. coli* was exposed to a plasma flow without the quartz glass, although the sterilization effect was small under the exposure using quartz glass. From these results, it was clarified that *E. coli* is sterilized not only by UV irradiation of the argon plasma, whose emission wavelengths are 282 and 309 nm, but also by the chemical species [17].

It was also found that inactivation of *E. coli* results from destruction of the cytoplasmic and cell membranes, since the potassium leakage concentration of cytoplasmic material increases [17] and the cell height decreases under plasma exposure [18]. The interaction mechanism between the post-discharge flow and water surface has also been reported [19].

In the present study, the focus was on the identification of generated chemical species and clarification by experimental and computational methods of how these species are transported in the post-discharge flow from the viewpoints of flow and temperature fields, concentration distributions of NO, NO$_2$, CO and O$_2$, spectrum analysis, excited species distribution, probe current and number of ions.

### 2. Experimental methods

Figure 1 shows a schematic of the experimental setup. The microwave plasma torch (Adtec Plasma Technology, Atmospheric Pressure Microwave Plasma) consisted of a power source, a coaxial cable, a cavity, a quartz tube with an inner diameter of 10 mm and a gas supply system [16]–[18]. The nozzle exit of the quartz tube was set at $z = 0$ mm, which was 20 mm from the bottom of the cavity. The frequency of the microwave was 2.45 GHz and the input power was 400 W. The operating gas employed was argon (99.99%) under a gas flow rate of 11 min$^{-1}$. The torch generated microwave plasma in the quartz tube, which formed a post-discharge flow downstream of the nozzle exit.

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The optical analysis system consisted of an optical multichannel analyzer (Hamamatsu Photonics, PMA-11), a digital camera (Nikon, D50) with a lens (Micro-Nikkor 60 mm), a BT-CCD camera (Hamamatsu Photonics, C9299-02) having a lens (Nikon, UV-Nikkor 105 mm) with band-pass filters, the center wavelengths of which were 310 nm of full-width at half-maximum (FWHM) 10 nm, 337 nm of FWHM 3 nm and 451.9 nm of FWHM 1 nm, and a reflection reduction box, which allowed spectrum analysis and visualization of ultra-weak emissions from the post-discharge flow. The flow field was visualized by the reflection of a laser sheet injected from the side, as shown in figure 1. To reflect the laser light, liquid paraffin was fed into the nozzle exit, smoke being produced by the heat of the plasma flow. Gas temperature was measured with an E-type thermocouple having a diameter of 0.076 mm. Gas concentration was measured by an NO, NO$_2$ and CO concentration meter (Draeger, Multiwarn II) and an O$_2$ concentration meter (New Cosmos Electric, XP-3180E). Probe current, 3 mm in length and 0.3 mm in diameter, at +90 V was measured by an electrometer (Kethley, 6517A) capable of measuring 1 fA at the minimum. The number density of ions was measured by a Gerdien condenser-type ion counter (Universal, IC-1000-W), whose maximum range is $2.0 \times 10^7$ counts cm$^{-3}$. All the above data were obtained by at least three measurements, which were then averaged.

3. Computational methods

The flow and temperature fields were analyzed by an axisymmetric model under a steady condition, and the time evolution of the temperature field was analyzed by a plane model under an unsteady condition. The computational domain of the axisymmetric model was a cylindrical geometry 60 mm in length and 50 mm in radius, the number of grids being 4212 ($54 \times 78$), and that of the plane model was 200 mm in length and 100 mm in width, the number of grids being 7452 ($69 \times 108$), as shown in figure 2. These computational models were based on the following assumptions: (i) the flow of an ideal gas is continuous and laminar, (ii) gravity is considered, and (iii) radiation loss and absorption are not considered. Under the above-mentioned assumptions, the governing equations were conservation equations of mass, momentum and energy, and the equation of state. The inlet conditions of temperature and gas velocity at AB were based on experimental results and the gas flow rate. The boundary conditions for the outlet were the Neumann condition, the wall of the torch being non-slip and constant temperature being obtained experimentally. Computational analysis was performed by commercial CFD-ACE+ software (ESI-Group, France), which employs the SIMPLEC method [20].
Figure 2. Computational domains.

Figure 3. Photographs of the post-discharge flow for the exposure times of (a) 1/60 and (b) 10 s.

4. Results and discussion

4.1. Thermofluid characteristics

Figure 3 shows photographs of the post-discharge flow for different exposure times of (a) 1/60 s and (b) 10 s. The other exposure conditions for both cases were an ISO of 1600 and an f value of 3.5. The post-discharge flow was invisible to the naked eye, as shown in figure 3(a), in which the quartz tube can be seen. When the exposure time increased from 1/60 to 10 s, the post-discharge flow was visualized, as shown in figure 3(b). The jet-like flow, colored light magenta, formed at the exit of the nozzle, but changed to dark blue toward the fringe of the flow, indicating that mixing between argon and air occurred in the region where the color changed to blue.

The flow field of the post-discharge, figure 4(a), was visualized experimentally and analyzed computationally. Flow velocity distribution, figure 4(b), and flow vector distribution, figure 4(c), were obtained by the axisymmetric model. The flow formed a small jet-like flow, whose tip was located at around $z = 25$ mm, and then traveled upward along the outside of the quartz tube and the bottom of the cavity. Finally, the flow traveled along the side of the cavity. The computational results agreed with the experimental results. The surrounding atmospheric air flowed toward the post-discharge flow and mixed with argon.

Correspondingly, a high-temperature region extended to $z = 25–30$ mm downstream, as shown by the experimental result presented in figure 5(a) and the computational result in...
Figure 4. Visualization of the flow field of the post-discharge flow. (a) Flow field visualized by laser sheet and liquid paraffin as a tracer. (b) Computational flow velocity distribution. (c) Computational flow vector distribution.

Figure 5. Temperature distributions for (a) experimental results and (b) computational results.

Figure 5(b), which was obtained by the axisymmetric model. The maximum temperature of 877 K was observed at the center of the nozzle exit, i.e. $x = z = 0$, and then the temperature rapidly decreased to about 300 K at around $z = 30$ mm. The high-temperature region expanded around the post-discharge flow since the main flow traveled upward due to buoyancy, most heat of the flow being transported upward. Therefore, it was possible to generate a low-temperature post-discharge flow downstream in such a short distance of 30 mm. Thus, the reason as to why a wider expansion was observed in the case of the experimental result was oscillation of the post-discharge flow from side to side, as shown in figure 6, which was obtained by the plane model. When the flow traveled to one side, the high-temperature region expanded widely toward the same side. Since each of the temperature data was obtained by averaging 100 continuous points for 10 s, the experimental result was more widely expanded than the result of the axisymmetric model.
4.2. Chemical species characteristics

Figure 7 shows the axial distributions of NO, NO$_2$, CO and O$_2$ concentrations. The O$_2$ concentration increased gradually from the nozzle exit and then rapidly increased at $z = 25–30$ mm, and finally became the same as that of air. This means that argon gas reached $z = 30$ mm and that convection transport was the most important factor for heat transport since this location is exactly the same as the location where the temperature decreased down to room temperature. The NO concentration of 15 ppm at the center of the nozzle exit decreased downstream and became zero at around $z = 35$ mm. The CO concentration showed the same tendency as that of NO. The NO$_2$ concentration increased from $z = 0$ to 5 mm and became constant at about 4 ppm until around $z = 30$ mm. The NO$_2$ concentration gradually decreased between $z = 30$ and 40 mm, the location being under the tip of the main flow. This result implies that diffusive transport is also an important factor in the transport of chemical species. It increased near the nozzle exit because the generation of NO$_2$ heavily depended on the NO and N concentrations [21]. NO was generated in the quartz tube since the NO concentration at $z = -20$ mm was 7.5 ppm, although the NO$_2$ concentration was 0 ppm in the quartz tube. This result might have been caused by the diffusive transport of N and O generated in the post-discharge flow in the upstream direction. Also, most chemical species were considered to be transported upstream outside the quartz tube by convection transport.
4.3. Optical emission characteristics

Figure 8 shows the emission line analysis of the post-discharge for (a) 200–860 nm and (b) 250–500 nm, the latter being a magnification of figure 8(a). Emission was observed in the horizontal direction to avoid injection of light from the quartz tube, as shown in figure 8(a). Emission lines from the excited argon atoms, i.e. Ar I, as well as from the N$_2$ second positive system and OH were observed. We previously identified generation of the OH radical in the microwave plasma of this torch since emission wavelengths of 282 and 309 nm were observed. Therefore, we consider the emission wavelength of 309 nm to be that of the OH radical. The emission wavelength of 452 nm is considered to be the Ar I line because the emission lines of the N$_2$ first negative system were not observed, except for 452 nm. The axial distributions of emission intensities for Ar I (811.5 nm), the N$_2$ second positive system (337.1 nm) and OH (309 nm) [22, 23] are shown in figure 9. The intensity of Ar I decreased immediately downstream of the nozzle exit, although the intensities of the N$_2$ second positive system and OH decreased gradually. Photographs taken through band-pass filters whose center wavelengths were 451.9, 337 and 310 nm, as shown in figure 10, correspond with the results in figure 9, the emission profiles of the excited species being obviously different. The Ar I emission of 452.2 nm was observed around the corner of the quartz tube, that is, most of the excited argon atoms seemed to travel upstream immediately and then disappear. Therefore, the intensity of Ar I decreased immediately, as shown in figure 9. The emission profile of the N$_2$ second positive system formed a jet-like shape, which was similar to that of the photograph shown in figure 3(b). The brightness around the center of the nozzle exit was lower because the mixing of argon and
4.4. Electrical characteristics

Figure 11 shows (a) the distribution of the probe current and (b) the characteristic curve of the probe current against the voltage applied to the probe. The maximum probe current of 0.6 $\mu$A
Figure 11. Probe current distribution at $V_p = +90$ V.

Figure 12. Axial distributions of (a) difference of between the number of positive and negative ions and (b) the number of positive and negative ions.

at the center of the nozzle exit decreased downstream and became zero at around $z = 20$ mm. This tendency corresponds to the axial distribution of the intensity of the $N_2$ second positive system, as shown in figures 9 and 10. The probe current suggests that electrons exist in the post-discharge flow since the probe current characteristic showed the same tendency as that
of the current–voltage characteristic of the Langmuir probe. Correspondingly, the number of negative ions was greater than that of positive ions from the nozzle exit to $z = 20$ mm, as shown in figure 12(a). The difference between the number of negative and positive ions decreased and its polarity became positive at $z = 30$ mm, although the difference was very small. However, the negative and positive ions were transported downstream below the main flow, i.e. $z = 30$ mm downstream, as shown in figure 12(b). These ions were considered to reach there by diffusive transport.

5. Conclusions

A post-discharge flow in atmospheric air was investigated to clarify the generation and transportation of chemical species that are considered to result in inactivation of bacteria. The flow, which was characterized by ultra-weak emission, could be visualized using optical analysis systems. The main flow traveled around the quartz tube as clarified by the profiles of temperature and velocity. The emission lines of Ar I, the N$_2$ second positive system and OH were mainly observed by spectrum analysis. NO$_2$ and a small amount of ions were transported downstream below the main flow. These results imply that most of the heat and chemical species were transported by the convective transport of the main flow but that a small amount of chemically active species ions might have been transported by diffusive transport further downstream, these species being considered to result in the inactivation of bacteria.

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