

# Inactivation of *Escherichia coli* in Small-Diameter Tubes by Remote Plasmas

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**ABSTRACT:** Heat and chemical treatments are commonly employed as decontamination methods in the medical industry. However, there are limitations to these methods, and new decontamination techniques are in demand. Among the state-of-the-art techniques, atmospheric-pressure plasma treatment is considered the most promising approach. Reactive radicals and ultraviolet light, generated in the plasma, are used to kill bacteria located away from the plasma source. In this study, we examined the inactivation of *Escherichia coli* samples, placed inside a long flexible tube of a small diameter, with plasma jet injection. Depending on the sample position, the inactivation effects of the radicals and ultraviolet light change significantly. Our experimental results show that long-life radicals are effective in plasma treatment. Moreover, the effectiveness of radicals in plasma decontamination was established using mutant gene strains without resistance to specific reactive radicals.

**KEY WORDS:** atmospheric-pressure plasma, plasma jet, catheter tube, *Escherichia coli*, decontamination, PVA-KI

## I. INTRODUCTION

Several decontamination methods such as heat, chemical, and radiation treatments are used in medical applications. Among these, atmospheric-pressure plasma irradiation is characterized by high chemical activities and apply low heat loads on the irradiated samples.<sup>1,2</sup> Previously, inactivation of harmful microorganisms using dielectric barrier discharge plasma<sup>3</sup> or glow discharge plasma<sup>4</sup> at atmospheric pressure have been actively studied. Despite the advances in decontamination techniques, microbial contamination remains a major concern, highlighting the need for new decontamination techniques. For example, conventional decontamination methods are difficult to apply to tubes with small diameters such as catheters. Plasma treatment has recently attracted significant attention owing to several advantages over the conventional bacteria inactivation methods.<sup>5–9</sup> Compared with heat decontamination, plasma treatment generates only a small amount of heat, and thus, degeneration of bio-materials and organic materials can be avoided. Moreover, plasma treatment is also devoid of residual chemical generation, which cause secondary health issues.

In this study, we investigated plasma decontamination inside flexible polymeric tubes of small diameters using an atmospheric-pressure plasma jet.<sup>10</sup> We confirmed that

plasma treatment produces active oxygen species such as hydroxyl radicals ( $\text{OH}\cdot$ ). The plasma source used in this study yielded  $\text{OH}\cdot$  radicals at lower electric powers compared with those used in previous works.

We used polyvinyl alcohol–potassium iodide (PVA-KI), which is synthesized using partially saponified PVA and KI, as a chemical probe owing to its ability to change color in the presence of reactive oxygen species. In our laboratory, PVA-KI was used to visualize the production and transport of radicals during the interaction between the atmospheric-pressure plasma jet and the solution. The reproducibility of the PVA-KI color reaction depends on this condition.<sup>11</sup> Therefore, we aimed to visualize the movement of reactive oxygen species in long tubes by observing the color change of PVA-KI. We confirmed that active oxygen radicals are crucial in heat decontamination by experimenting with mutant gene strains. Several bacteria samples with varying resistances against different radical attacks were prepared via gene manipulation. These samples were used to compare the inactivation speeds and to evaluate the effectiveness of various radicals in plasma decontamination.

## II. MATERIALS AND METHODS

### A. Strain

The bacterial sample used in this study was *Escherichia coli* NBRC106482. In addition, we prepared four mutant samples (Table 1) for the experiments. The first sample (wild-type) was a wild strain of normal bacteria. The second sample ( $\text{Sod}^-$ ) was a mutant strain<sup>12</sup> without three enzymes (*sodA*, *sodB*, and *sodC*), and thus, this strain was not resistant against the superoxide radical  $\text{O}_2^-$  attacks. The third sample ( $\text{Hpx}^-$ ) was a mutant strain without three enzymes (*katG*, *katE*, and *ahpCF*) and was not resistant against hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) attacks.<sup>13,14</sup> The fourth sample ( $\text{Sod}^-$  and  $\text{Hpx}^-$ ) was a mutant strain and not resistant to both superoxide and peroxide radicals.

### B. Media and Culture Conditions

The *Escherichia coli* samples were cultured at 30°C for 18 h in a liquid medium (EM9, with 0.1% casamino acid). The bacterial liquid was then centrifuged to remove the

**TABLE 1:** Strain with mutant gene and its genotype

Strain	Genotype	Resistance for ROS	
		$\text{H}_2\text{O}_2$	$\text{O}_2^-$
WT	<i>E. coli</i> NBRC 106482	+	+
$\text{SOD}^-$	$\Delta\text{sodA}$ , $\Delta\text{sodB}$ , $\Delta\text{sodC}$	+	–
$\text{HPX}^-$	$\Delta\text{katG}$ , $\Delta\text{katE}$ , $\Delta\text{ahpCF}$	–	+
$\text{SOD-HPX}^-$	$\Delta\text{sodA}$ , $\Delta\text{sodB}$ , $\Delta\text{sodC}$ , $\Delta\text{katG}$ , $\Delta\text{katE}$ , $\Delta\text{ahpCF}$	–	–

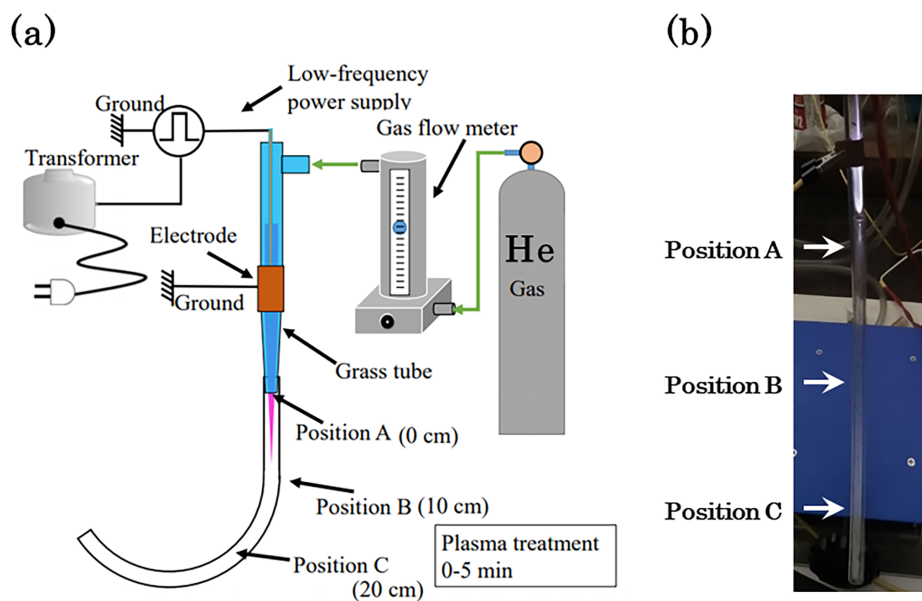
supernatant and was suspended in a 0.1% polypeptone–water solution. The treated solution was then filtered using filter paper.

### C. Experimental Setup

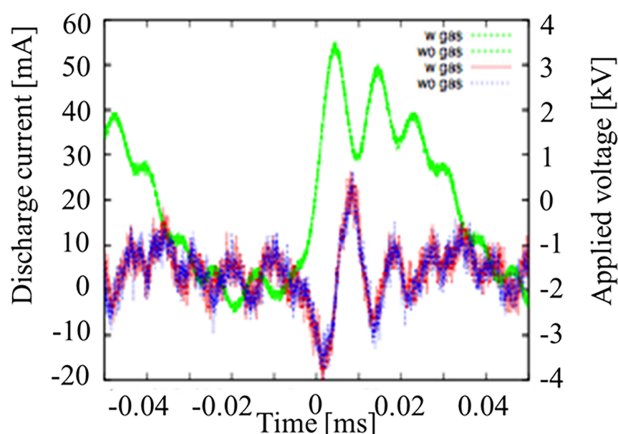
The plasma source used in this study comprised a low-frequency power supply and glass tube.<sup>15</sup> Figure 1 shows the experimental setup, and Fig. 2 shows the waveform, applied voltage, and discharge current. Helium gas was supplied through a glass tube, and an electric power was applied to a needle electrode using a commercial power source (Loggy Electronics Ltd LHV-13AC).<sup>16</sup> The flow rate of the helium gas was set to  $\sim 5$  L/min, and the input electrical power to LHV-13AC was  $\sim 15$  W, which was lower than that used in a previous work.<sup>10,17</sup> Two flexible tubes, 20 cm (tube 1) and 50 cm (tube 2) in length were connected to the glass tube. Filter papers containing bacteria were wound around cotton strings, which were then inserted into the tube connected to the glass tube, and then, the plasma jet was introduced into the tube for 0–5 min.

### D. Inactivation of *E. coli* in a Long Narrow Tube

Three positions of the paper filters are shown in Fig. 1. At position A (exit of plasma source), the samples are directly irradiated by the plasma. The plasma jet barely reaches



**FIG. 1:** Experimental setup with a long tube (tube 2). The flow rate of the helium gas is 5 L/min, and the electrical power is 15 W. (a) Drawing of electrode configuration and gas flow lines; (b) photo of the measuring position in the tube.

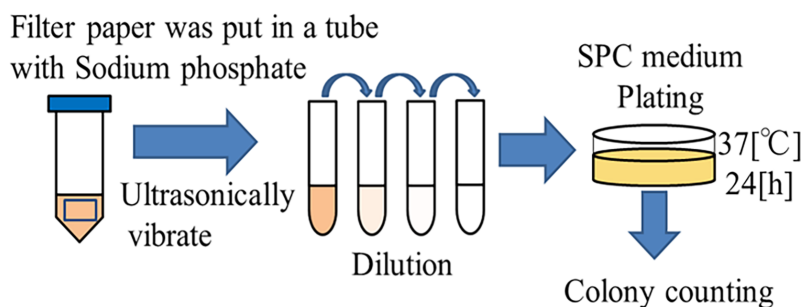


**FIG. 2:** Waveform of the applied voltage and discharge current

position B (10 cm from plasma source), and at position C (20 cm from plasma source), only helium gas flows at room temperature. The dilution flow and colony counting are shown in Fig. 3. After the irradiation, the filter paper was placed in 1 mL of the recovered solution, and the bacteria was extracted using an ultrasonic shaker. The bacterial liquid was diluted and smeared on agar and then cultured at 37°C. After 24 h, the generated colonies were analyzed to obtain the number of bacteria in colony forming units (CFUs)/mL.

### E. Visualization of the Reach of Radicals in the Tube

We observed the transport of radicals inside the polymer tube using a PVA-KI gel. The PVA-KI mixture comprised 50 mL of PVA (equivalent to 20%), 50 mL of water mixed with 5 g of KI 12 (equivalent to 5%), and 5 g of borax (equivalent to 2%). The mixture was then microwaved for 15 s three times, and subsequently, 200 mL of water was added to 10 mL of the PVA mixture (equivalent to 4%). Finally, water was added to obtain a



**FIG. 3:** Flow of extract, dilution, plating, and colony counting after plasma irradiation

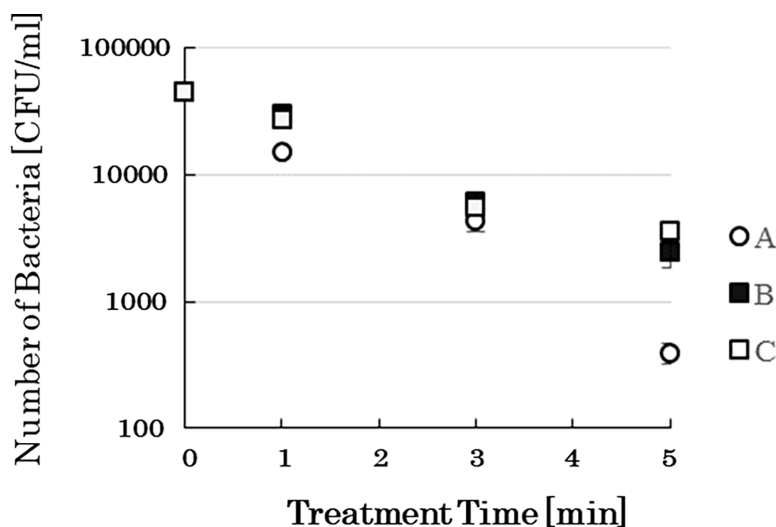
total mixture volume of 250 mL via stirring. The PVA-KI gel adhered to the inner wall of tube 1 during the testing of the transport of active oxygen in the long tube (Fig. 4a).

The experimental conditions were as follows. Flow rate of He gas: 2 L/min; power supply voltage: 4 kV; polymer tube length: 23 cm; PVA-KI gel: PVA 20%, KI 5% w/v, and water 80%. The irradiation times were 30, 60, and 120 s for the positions A, B, and C, respectively.

### III. RESULTS AND DISCUSSION

#### A. Inactivation of *E. coli*. at Each Position in the Tube

Figure 4 shows the results obtained using the 20-cm-long tube 1, and the inactivation effect is compared with the treatment time for the samples at positions A, B, and C. Evidently, the number of bacteria decreases with treatment time. With a treatment time of five minutes, the bacterial count in the sample at position A decreased by two orders of magnitude. The bacterial count for the samples at positions B and C were approximately equal and decreased by one order of magnitude after the same treatment time. The bactericidal effect at position A was due to ultraviolet light and short-life radicals. Furthermore, only long-life radicals contributed to the decontamination at positions B and C. The effect of heat can be neglected as the gas temperature was maintained at room temperature or lower.

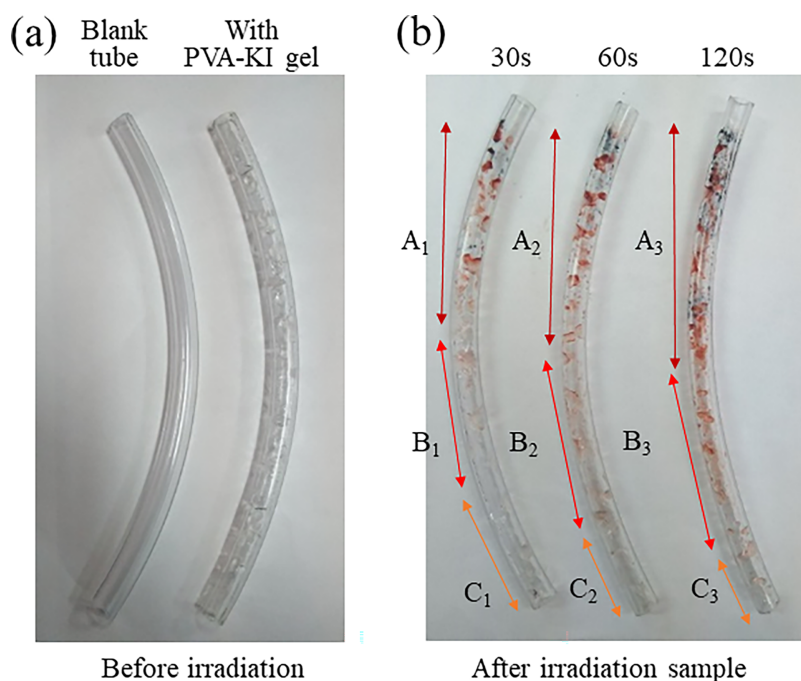


**FIG. 4:** Comparison between the bactericidal effects at each position and different treatment times. Tube 1 is used (20 cm). Marker A denotes the exit of the plasma source, B is located 10 cm away from the plasma source, and C is located 20 cm away from the plasma source.

## B. Visualization of the Reach of Radicals in the Tube

We classified three areas of radical transport, viz. A, B, and C, inside the tube (Fig. 5b). In the areas denoted by A1, A2, and A3, the samples appear red and blue, representing the transport of a large number of radicals; these areas may affect the heating temperature. In the areas represented by B1, B2 and B3, the colors of the samples are less intense than those of the samples in A. This low color intensity is observed, because the quantity of radicals reaching these areas decrease with longer transportation times. In the areas marked as C1, C2 and C3, the samples exhibit a light-red color, the intensity of which is weaker than that in the samples in B. This weakly intense red color indicates that the radicals react with the PVA-KI adhered to the tube wall in A and B before arriving at C.

From these results, we can conclude that although a longer tube reduces the number of radicals reaching the extreme end of the tube, longer irradiation times offset this reduction by a significant amount. Thus, the trajectory of a plasma jet traversing a tube can be visualized via radical production and transportation monitoring using PVA-KI as well as *E. coli* at different positions inside the tube.



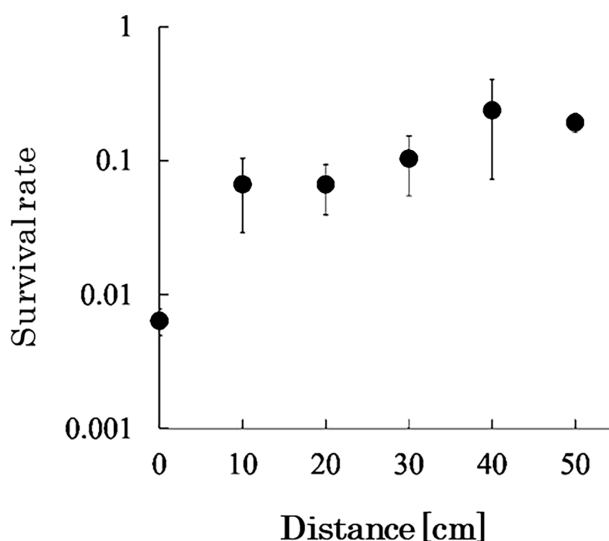
**FIG. 5:** (a) Experimental setup for visualizing radical transport in a long tube. (b) Plasma irradiation results of PVA-KI. The color of the PVA-KI gel changes when it reacts with the reactive oxygen species. In area A, A1 = 2–8 cm, A2 = 8–15 cm, and A3 = 15–23 cm. In area B, B1 = 2–10 cm, B2 = 10–17 cm, and B3 = 17–23 cm. In area C, C1 = 2–13 cm, C2 = 13–19 cm, and C3 = 19–23 cm.

### C. Inactivation of *E. coli* in the Longer Tube

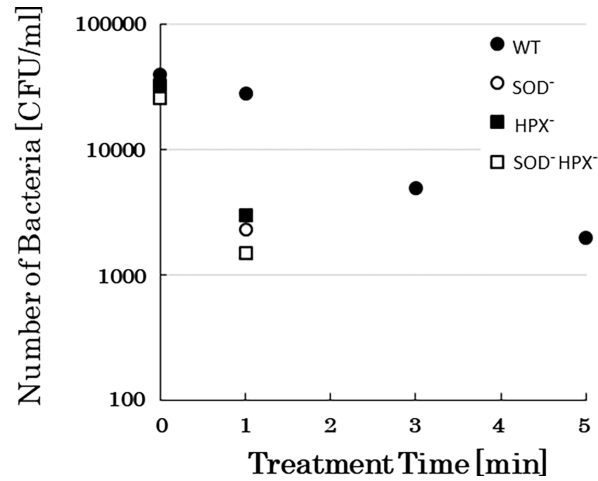
In Figure 6, which displays the results obtained for tube 2, the vertical axis denotes the survival rate as a function of the sample position. This figure indicates the bacterial count after five minutes of treatment. The survival rate at position A (0 cm) is minimum and increases significantly until position B (10 cm). However, a constant survival rate of  $\sim 0.1$  is maintained for the long distance travel of the species, because the sample around position A is sterilized by the combined use of ultraviolet light and short-life radicals ( $\text{OH}\cdot$ ). In addition, the remote samples are mainly sterilized by long-life radicals. The data show that inactivation by long-life radicals can be sustained over long distances. This observation for tube 2 also confirms that a plasma jet traveling through a tube can be visualized by monitoring the radical production and transportation using PVA-KI as well as *E. coli* at different positions inside the tube.

### D. Survivability of Reactive Oxygen Species Degrading the Enzyme Mutant Strains of *E. coli* under Plasma Irradiation

Figure 7 shows the results of remote plasma treatment (inactivation experiment) with the wild and mutant gene strains, performed using a low-volume vessel. Evidently, the number of wild strains can be reduced by 1/10 with five minutes of treatment. Thus, the corresponding D-value is five minutes. We hypothesize that a perfect decontamination can be realized by increasing the treatment time.



**FIG. 6:** Comparison between the bactericidal effect at each position (0–50 cm). The treatment time is 5 min, and tube 2 is used (50 cm).



**FIG. 7:** Comparison between the bactericidal effect for each gene: *E. coli* wild-type, Sod<sup>-</sup>, Hpx<sup>-</sup> and Sod<sup>-</sup> and Hpx<sup>-</sup> at position B

The mutant gene strain without anti-oxidization enzymes was inactivated in a significantly shorter time, and the corresponding D-value is within one minute. After two minutes of treatment, the bacterial count becomes lower than the detection limit. The three mutant strains exhibit similar behavior. These results suggest that two reactive oxygen species  $O_2^-$  and  $H_2O_2$  are involved in the remote plasma treatment of long flexible tubes. The decontamination results (D-value) for the four strains are summarized in Table 2.

The experimentally visualized radical transport in the longer tube indicates that the flux of radicals traversing a longer tube is significantly smaller than that traversing a shorter tube. However, this flux reduction can be countered and, the radical flux traversing a longer tube can be increased by increasing the plasma irradiation times.

#### IV. CONCLUSIONS

Plasma decontamination of long flexible tubes was conducted around the plasma injection point and inside the whole tube, and we found that *E.coli* can be successfully

**TABLE 2:** D-value of the plasma irradiation on *E. coli*

Strain	D value [min]
WT	3.75
SOD <sup>-</sup>	0.67
HPX <sup>-</sup>	0.70
SOD-HPX <sup>-</sup>	0.65

inactivated using this proposed decontamination method. Atmospheric-pressure plasma can remotely inactivate pathogens inside flexible tubes, primarily because of long-life radicals, which are generated in the plasma source and carried by the flowing helium gas. This method can be used to treat more than 50 cm long tubes.

The plasma-treatment D-value for the gene mutant strains ( $\text{Sod}^-$ ,  $\text{Hpx}^-$ ) were noticeably smaller than that for wild strains, indicating that  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  play crucial roles in remote plasma treatment of bacteria. A comparison of these results with those of the mutant strain confirmed that long-life radicals ( $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$ ) are critical to the inactivation process.

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