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Effects of H_2O_2 and Low pH Produced by Gliding Arc Discharge on the Inactivation of *Escherichia Coli* in Water

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ABSTRACT: The efficacy of gliding arc (GA) discharge for the generation of hydrogen peroxide (H_2O_2) and water with a low pH was studied because H_2O_2 combined with low-pH environment is known as a strong oxidizer that can be used for the bacterial inactivation. The ability of the GA discharge to inactivate *Escherichia coli* in water was tested experimentally, and the inactivation was found to increase with the plasma treatment time and rate of water injection flow to the GA discharge system. The best result showed a 2-log reduction of the number of colony-forming units of *E. coli* from 10^4 to 10^2 at a water injection flow rate of 180 mL/min. Furthermore, pH in the plasma-treated water was decreased from 6.0 to 3.55 after 25 min of treatment.

KEY WORDS: hydrogen peroxide, gliding arc discharge, *E. coli*, bacterial inactivation in water, pH variation

I. INTRODUCTION

Plasma sterilization is one of the emerging technologies in the biomedical field[1] and has been applied to the inactivation of microorganisms in water.[2–5] The sterilization has been attributed to the ability of plasma discharges to generate active plasma species, for example, hydroxyl (OH), oxygen, ozone, hydrogen peroxide (H_2O_2), ultraviolet, and electric fields.[6–8] Each of these plasma species may play a role in the inactivation of microorganisms. Although most of these active species have a very short half-life on the order of microseconds or less,[9–12] H_2O_2 and ozone have a relatively long half-life, so they may be useful in the treatment of a large volume of contaminated water.[13–15] Ozone has to be produced in air and then injected to water.[16,17] On the other hand, H_2O_2 can be produced directly from the dissociation of water molecules by plasma discharge,[6,15,18,19] and its concentration in water can last for a relatively long time, that is, more than 10 minutes.[15,20]

A gliding arc (GA) discharge can generate a large amount of active plasma species because of the unique plasma properties and gas flow inside a GA generator. The maximum concentration and effective time of H_2O_2 generated by the GA discharge in water and the capability of bacterial inactivation was reported in a previous study.[20]

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The GA discharge can be defined as an auto-oscillating periodic discharge between at least 2 diverging or nondiverging electrodes propelled by a gaseous flow,[6] resulting in a high degree of nonequilibrium to sustain a selective chemical process.[21] The first GA discharge, known as Jacob's ladder, was invented about 100 years ago; it used 2 flat, vertical, two-dimensional electrodes whose distance was increased along the axial direction.[6,22,23] The GA discharge has the benefits of both equilibrium and nonequilibrium discharges because of the combination of the powerful and energy-efficient transitional discharge.[6,21–23] The arc discharge first ignites as a thermal plasma at the smallest gap between the 2 electrodes. Then the arc is forced to move downstream by a stream of gas and is convectively cooled by the stream of room-temperature gas, after which it becomes a nonequilibrium discharge during the space-time evolution.

To increase the residence time for a higher degree of the completion of chemical reactions and provide an intensive convective cooling of the discharge zone, a transitional GA discharge using cylindrical tube geometry in a reverse vortex (i.e., tornado) flow was developed and introduced by a number of researchers.[6,21–27] The reverse vortex flow provides excellent thermal insulation of active species from the cylindrical wall, significantly reducing energy loss to the surroundings and thus increasing its energy efficiency. Another benefit is that the residence time of gas to be treated by plasma is relatively long, a desirable phenomenon for various chemical reactions. So, the rotating GA discharge provides the ability to both increase specific power input and ensure uniform treatment of gas.[23,26]

The GA discharge was reported to dissociate water molecules, including the recombination process of hydroxyl radicals, leading to the formation of H_2O_2.[20] However, it is not clear whether the GA discharge could provide sufficient inactivation power for the treatment of a large volume of water. Furthermore, it is not clear how low the plasma discharge can reduce the pH of water for bacterial inactivation.[5,28]

Hence the objective of this study was to investigate whether the GA discharge could generate a significant quantity of H_2O_2 and a significant reduction in the pH of water, thus providing good antimicrobial properties[24,29] for the treatment of a large volume of water. For this purpose, the study investigated the optimum condition for water and gas flows into the GA discharge system to obtain the maximum H_2O_2 concentration and the minimum pH in water. In addition, the study investigated the synergistic effects of H_2O_2 and low pH on the sterilization of contaminated water as a function of plasma treatment time.

II. METHODS

The experimental setup used in this study is illustrated in Fig. 1. The test setup consisted of 3 major parts: the first part consisted of 2 identical GA discharge systems, each driven by its own power supply; the second to handle both air and water flows, that is, to provide controlled flows of gas and water to the GA discharge system; and the third to contain 20 L of bacteria-contaminated water and to receive plasma-treated liquid and gas. The basic approach in the study was to have both air and distilled water pass

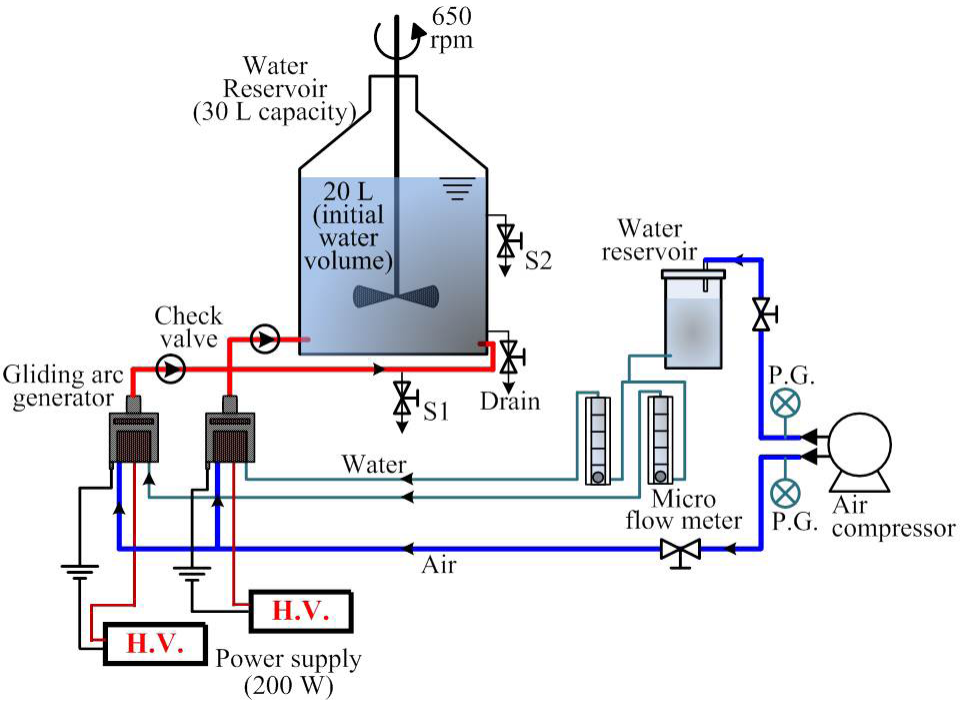


FIGURE 1. Experimental setup for the plasma water treatment using gliding arc discharge. H.V., high-voltage electrode; P.G., pressure regulator; S1, first sampling port; S2, second sampling port.

through the GA discharge system first and then introduce the plasma-treated water to a large volume of contaminated water for the inactivation of microorganisms. To provide gas flow to the 2 GA generators, an air compressor was used, and the air flow rate was controlled with a valve and a pressure gauge. The compressed air also was sent to the top of the water reservoir, as shown in Fig. 1, so that water could be pushed out the bottom of the reservoir through an exit to the GA generator at a uniform flow rate, which was monitored by a micro flow meter.

Figure 2 shows a sketch of the plasma discharge system. To use an excellent energy density for chemical reaction and desirable residence time of gas in the GA discharge, this study used a 3-dimensional GA system with 2 circular disk electrodes with a gap of 2.5 mm. The 2 electrodes were connected to a power supply that delivered 200 W at a maximum voltage of 3 kV. Compressed air was introduced tangentially to the gap space at the outer edge of the 2 circular electrodes through 6 small nozzles (① in Fig. 2; diameter = 0.5 mm) such that the arc discharge could move along the circumference of the electrodes, resulting in a gliding arc. Both electrodes were made of stainless steel and protected by the insulating material (④ in Fig. 2) polytetrafluoroethylene (Teflon) for safe operation. Water also was injected tangentially through 6 small nozzles (③ in Fig. 2; diameter = 1.5 mm) to the plasma arc jet exiting from a nozzle (② in Fig. 2; di-

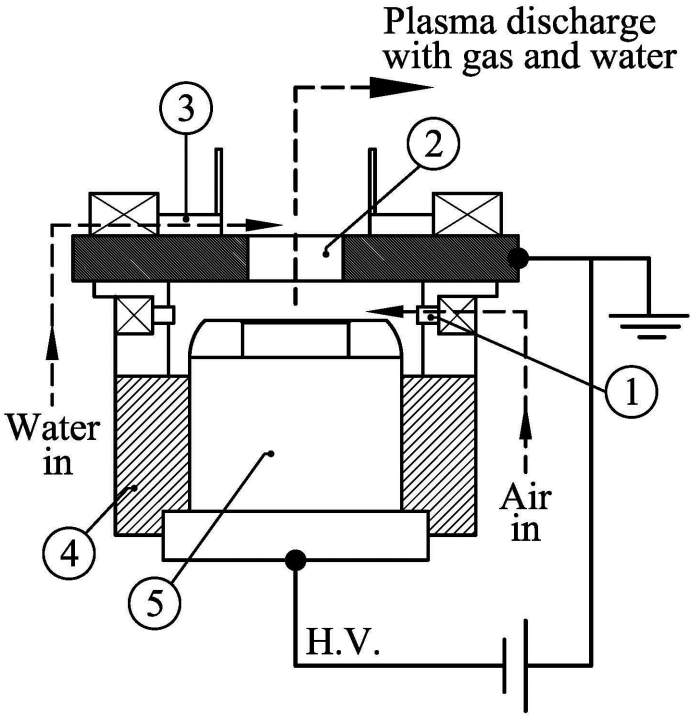


FIGURE 2. Sketch of gliding arc (GA) generator: ①, 6 nozzles for tangential entry of air flow to the gap between 2 electrodes and ground electrode; ②, nozzle for the exit for GA jet, ground electrode; ③, 6 nozzles for the tangential injection of water to GA discharge; ④, Teflon insulator protecting high-voltage electrode (H.V.); ⑤, H.V. (negative voltage).

ameter = 9.5 mm) positioned at the center of the ground electrode. Of note is that water did not make contact with the high-voltage (HV) electrode (⑤ in Fig. 2).

A reservoir with a maximum capacity of approximately 30 L was connected to an outlet port at the GA discharge system. After the injected water reacted with the plasma jet inside the GA discharge system, both gas and the plasma-treated water entered the water reservoir, which contained an initial volume of 20 L of contaminated water, through a 30-cm-long flexible plastic tube. In the middle of the flexible tube, a check valve was installed to prevent the back flow of water to the HV electrodes. After each test, the water reservoir was cleaned repeatedly with both sulfuric acid and tap water and then dried in a fume hood. Then, the water container was filled with 20 L of pure deionized water for the next test, and the flexible tube from the GA system was reconnected to the reservoir. The plasma-treated water exiting from the GA system was mixed with bacteria-contaminated water at the reservoir, while the plasma-treated gas passed through the water inside the reservoir.

The concentration of H_2O_2 in water was measured using a peroxide test strip (EMD Chemicals/Merck, Darmstadt, Germany) by observing the change in the color of the

Water Injection flow rate (mL/min)	20	40	80	120	140
Concentration (mg/L)	100	> 50	> 50	30	25
Color changes in peroxide test strips					

FIGURE 3. Variations of H_2O_2 concentrations at different water injection flow rates and air flow rate of 3.8 standard cubic feet per minute in the gliding arc discharge system. Note that the H_2O_2 concentration was measured in plasma-treated water before it was mixed with 20-L water at the reservoir.

strip. The protocol for use of the peroxide test strips was as follows: 50 mL samples of plasma-treated water were collected, and a test strip was dipped into each water sample for approximately 15 seconds. Upon removal from the water, the change in the color of the test strip was observed and immediately recorded with a camera and compared with the colors from the calibration standard data provided by the manufacturer.

The flow rate of water to the GA system varied from 20 to 180 mL/min to examine the effect of the water flow rate on the concentration of H_2O_2 at a uniform air flow rate of 3.8 standard cubic feet/min. In conjunction with the above experiments, tests were performed to verify the inactivation effect of H_2O_2 generated by plasma discharge. *Escherichia Coli* was used for inactivation experiments. Cultures of *E. coli* were grown in an incubator for 18 hours at 37°C and diluted using a most probable number method[30,31] to get a range of concentration of 10^3 to 10^5 colony-forming units (CFUs)/mL as initial test conditions. The number of CFUs in all water samples were estimated using the aerobic heterotrophic plate counting (HPC) method.[32,33] For bacterial inactivation tests, cultures of *E. coli* were added to a reservoir filled with 20 L of distilled water. Immediately after adding *E. coli* to water, it was stirred continuously with an electrical stirrer (Eurostar/IKA, Staufen, Germany) at 650 rpm to uniformly distribute *E. coli* at the reservoir. After stirring water for 5 minutes, 50-mL samples were collected in a sterile tube for the measurement of CUFs in both control and inactivation tests.

In the control test (i.e., no plasma treatment; Fig. 4) *E. coli* was added to deionized water at the reservoir where uncontaminated deionized water passed through the GA system at a flow rate of 180 mL/min with the plasma power turned off.

For the bacterial inactivation test, 3 water samples, each 1 mL, were collected from each 50-mL water sample using a sterile pipette and spread on brain–heart infusion agar plate (Thermo Fisher Scientific, Waltham, MA) inside a clean bench within 5 minutes after collecting 50-mL water samples from the reservoir. Before spreading 1 mL of the samples on the agar plates, the 50-mL sample was stirred continuously with a vortex mixer (Genie 2, Thermo Fisher Scientific). All agar plates containing water samples were dried inside the clean bench for 30 minutes at room temperature and incubated for 18 hours at 37°C.[1] The number of CFUs was determined using a colony counter (Digital Colony Counter/HYC-560, Hanyang Scientific Equipment, Seoul, Korea). Before

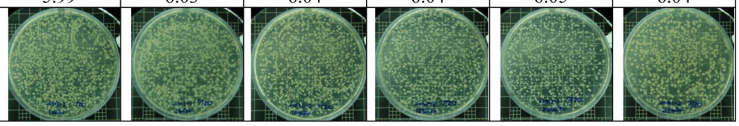
Control (No plasma treatment)						
Time (min)	0	5	10	15	20	25
cfu No./mL	10^5	10^5	10^5	10^5	10^5	10^5
pH	5.99	6.03	6.04	6.04	6.05	6.04
HPC image						

FIGURE 4. Control test: results of *Escherichia coli* inactivation experiment without gliding arc discharge but with a water injection flow rate of 180 mL/min. cfu, colony-forming unit; HPC, heterotrophic plate counting.

counting CFUs of *E. coli* on agar plates after incubation, the HPC images were recorded with a camera (Figs. 4–6). Measurements of H_2O_2 concentration were taken along with the measurements of the numbers of *E. coli* CFUs.

III. RESULTS

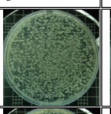
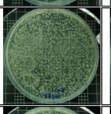
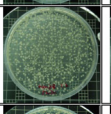
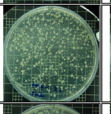
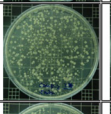
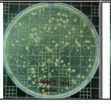
Figure 3 shows the results of H_2O_2 concentrations in plasma-treated water collected from the first sampling port (S1 in Fig. 1) at different water flow injection rates in the form of color changes on the peroxide test strips. As expected, the best result was found at the lowest water flow rate of 20 mL/min. With the lowest rate of water flow to the GA system, the maximum concentration of H_2O_2 in water was 100 mg/L.[20]

Before the inactivation test on *E. coli*, the control test was performed at the reservoir, where additional water of 9 L was introduced at a water flow rate of 2 × 180 mL/min for 25 minutes with the plasma power turned off. Figure 4 shows that the number of CFUs for control samples was maintained at 10^5/mL over 25 minutes. Even though the water volume of the reservoir was increased to 29 L because of the water added to the reservoir, there was no significant change in the resulting number of CFUs.

Figure 5 shows photographic images of the plates used in the HPC measurements of *E. coli* in the inactivation test. Significant levels of cultured *E. coli* were observed by the HPC image visualization in water collected from the reservoir when the water injection flow rate to the GA systems varied from 0 to 100 mL/min. The initial CFUs from the control samples varied at different water injection flow rates, which were maintained $\geq 10^4$ CFU/mL. The serial dilutions of samples were used to measure CFUs. For the cases with a water injection flow rate >140 mL/min, the number of CFUs per milliliter significantly decreased at a plasma treatment time of 10 minutes (Fig. 5B). The number of CFUs consistently was reduced with increasing water injection flow rates (from 140–180 mL/min) to the GA system. In summary, the ability of bacterial inactivation in water improved as the amount of water injection and plasma intensity (accumulated over time) increased.

Figure 6 shows the CFU results as a function of the plasma treatment time. As the rate of water flow to the GA system was increased from 120 to 140 mL/min, the con-

A

Water injection (mL/min)	No Treatment	Plasma Treatment			
	Initial cfu/mL				
Time (min)	0	1	4	7	10
0	10^5				
20	10^5				
40	10^4				
60	10^4				
80	10^4				
100	10^3				

B

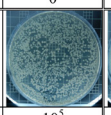
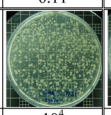
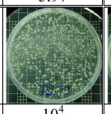
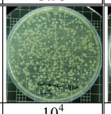
Water injection (mL/min)	No treatment	Plasma Treatment					
Time (min)	0	4	10	13	16	20	25
120							
cfu No/mL	10^5			1023 ± 21	810 ± 11	709 ± 07	449 ± 13
pH	6.11	4.39	3.96	3.85	3.77	3.69	3.61
140							
cfu No/mL	10^4			728 ± 32	576 ± 16	474 ± 0	300 ± 40
pH	5.97	4.2	3.84	3.73	3.67	3.61	3.55
160							
cfu No/mL	10^4			570 ± 50	474 ± 22	388 ± 10	246 ± 14
pH	5.95	4.25	3.89	3.8	3.74	3.67	3.61
180							
cfu No/mL	10^4			631 ± 2	435 ± 34	426 ± 94	167 ± 18
pH	6.15	4.26	3.85	3.77	3.69	3.63	3.55

FIGURE 5. A. results of *Escherichia coli* inactivation experiments at various water injection flow rates (from 0 to 100 mL/min) with an initial water volume of 20 L at the reservoir. **B.** Results of *E. coli* inactivation experiments and pH variations at various water injection flow rates (from 120 to 180mL/min) with an initial water volume of 20 L at the reservoir.

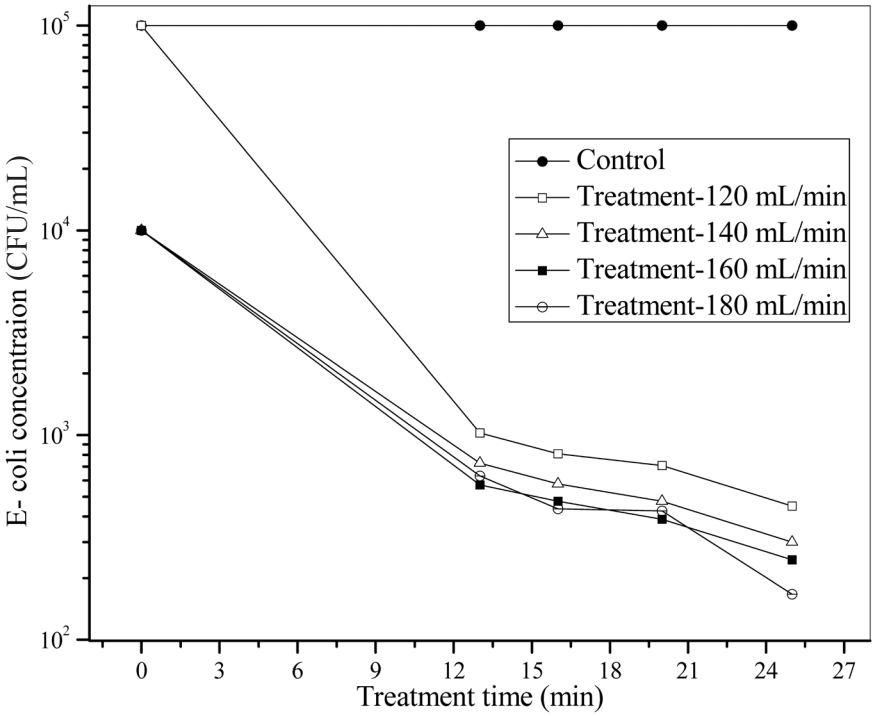


FIGURE 6. Results of *Escherichia coli* inactivation experiments at various water injection flow rates with an initial water volume of 20 L at the reservoir.

centration of *E. coli* in the form of CFUs was reduced dramatically. The initial number of CFUs was 10^5 CFU/mL for a water injection flow rate of 120 mL/min, whereas they were 10^4 CFU/mL for the other 3 plasma treatment cases. When the water injection flow rate was ≥120 mL/min, the CFU measurements showed a 2-log reduction after 13 minutes of plasma treatment. Furthermore, in all plasma-treated cases shown in Fig. 6, the number of CFUs consistently was decreased after 13 minutes of plasma treatment. For the case of the 2-log reduction for 13-minute plasma treatment, 15.6 kJ/L of energy was needed, which increased to 30 kJ/L for 25-minute treatment, with a 2.6-log reduction according to the result obtained at a water injection flow rate of 120 mL/min.

Figure 7 shows the variations of pH in the plasma-treated water collected in the reservoir at the different rates of water flow injection from 120 to 180 mL/min over the plasma treatment time. The pH of the plasma-treated water from the reservoir was decreased from the mean value of 6.05 ± 0.10 at $t = 0$ minutes to 3.58 ± 0.03 at $t = 25$ minutes, a phenomenon that can be attributed to the presence of an H^+ ion produced from water molecules dissociated by GA discharge.[5,6]

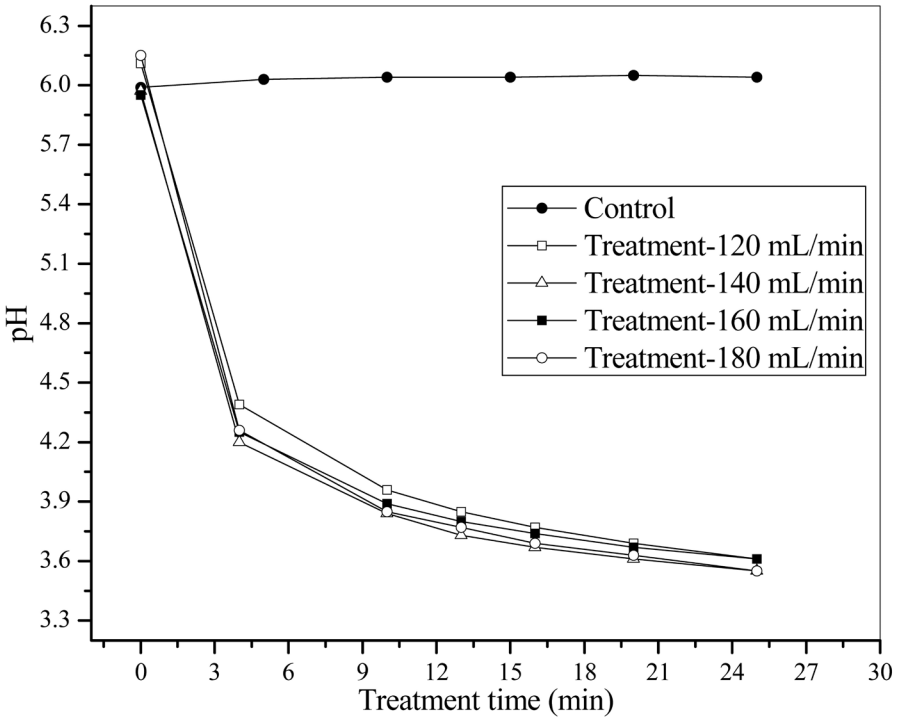


FIGURE 7. Results of pH changes at various water injection flow rates with an initial water volume of 20 L at the reservoir.

IV. DISCUSSION

This study reports the changes in both H_2O_2 concentration and pH in plasma-treated water with the GA discharge for the inactivation of microorganisms. Experimental results obtained in the study indicate that the GA discharge reacting with water could effectively generate H_2O_2 while it reduces the pH of water. When water is exposed directly to the GA discharge, the reactions can occur with the dissociation of water molecules as follows[6,15,18,19]:

$$e + H_2O \rightarrow H + OH + e^- \quad (1)$$
$$e + H_2O \rightarrow H^- + OH \quad (2)$$
$$M^+ + H_2O \rightarrow H_2O^+ + M \quad (3)$$
$$H_2O^+ + H_2O \rightarrow H^+(H_2O) + OH \quad (4)$$

Then, H_2O_2 can be formed from the recombination of hydroxyl radicals.[19,34]

$$OH + OH + M \rightarrow H_2O_2 + M \quad (5)$$

Note that in the case of zero water injection to the GA system, H_2O_2 was not produced,[20] indicating that water injection was necessary for the production of H_2O_2. This

strongly suggests that H_2O_2 must have been generated from the dissociation of water by the GA discharge. OH radicals produced by the GA discharge disappear fast and are quickly converted into H_2O_2. In other words, the generation of H_2O_2 includes the contributions from OH radicals and other active species that might have been formed by the GA discharge.

The presence of H_2O_2 is a reasonable indicator for the formation of hydroxyl radicals by plasma discharge with water.[15,18,24,35,36] H_2O_2 is formed as the final process of the combination of various radicals such as hydroxyl radicals.

In the overall set of reactions, the concentration of H_2O_2 in water can increase with plasma treatment. Furthermore, the pH of water decreased because of the presence of H^+ in Eq. (4), as shown in Fig. 7. In this study, a significant decrease in pH can be attributed to positive charges (M^+) created in the plasma discharge that reach the water molecules and exchange charges with them, resulting in the creation of H^+ ions,[15] as seen in Eqs. (4) and (5).

Results shown in Figs. 5 and 6 indicate the significant ability of the current plasma water treatment method to inactivate bacteria when H_2O_2 was combined with a low-pH environment. Note that neither H_2O_2 itself nor low acidic water are not strong oxidizers. However, H_2O_2 in acidic water becomes a very strong oxidizer and an effective tool for the inactivation of microorganisms, having a direct effect on the outer membrane of microorganisms because of the peroxidation of cell membranes.[2,15]

Although H_2O_2 was not generated in the case of no water injection to the GA discharge[20] (Fig. 5A, top row), it is possible that some *E. coli* was inactivated by means other than H_2O_2, most likely due to other reactive oxygen species such as superoxide (O_2^-) and peroxynitrite ($ONOO^-$).[5,6] An interaction between plasma-generated species may have damaged the cell membrane by physical or chemical modifications. For example, lipid peroxidation of membrane often leads to leaky cells.[37]

V. CONCLUSIONS

This study investigated the feasibility of a plasma water treatment system using a GA discharge system that can be used for the treatment of a large volume of water. The bacterial inactivation experiments with an initial water volume of 20 L were conducted at various water injection flow rates to the GA discharge system. The value of CFUs decreased with increasing plasma treatment time and water injection flow rate. Bacterial inactivation was effective at a water injection flow rate >120 mL/min. In addition, pH in the plasma-treated water decreased from 6.05 ± 0.10 at t = 0 min to 3.58 ± 0.03 at t = 25 min. H_2O_2 combined with low-pH water was found to be a strong oxidizer with a significant ability to inactivate bacteria. Future work should include capturing H_2O_2 lost during the gas phase through condensation, a process that may further improve the inactivation capability of GA discharge.

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