# Sterilization of Underwater Bacteria by Ozone Bubble Pulsed Discharge

Daito Ueda, Ryosuke Miyata, Rika Tsubota, Naoki Osawa, *Member, IEEE*, Ikuhiro Tanida, Satoshi Osawa, and Koichi Nakata

***Abstract*— Electric discharge is going to be applied to bactericidal inactivation in water without chemical dosing. In this article, the sterilization experiments of simulated reused water from an industrial washing machine were conducted by three different treatment methods: 1) air bubble pulsed discharge (ABPD); 2) $O_3$ alone; and 3) ozone bubble pulsed discharge (OBPD). The results showed that: 1) the highest sterilization effect was obtained by OBPD treatment and 2) since the number of sterilized bacteria by OBPD treatment was larger than the sum of the number of sterilized bacteria by ABPD treatment and that of by $O_3$ alone treatment, the synergistic effect by OBPD treatment exists. In order to clarify the decisive factor of synergistic effect by OBPD treatment, we measured dissolved $O_3$ concentration, pH, $NO_3^-$ concentration, emission intensities of O radical, and $H_2O_2$ concentration. As a result, dissolved $O_3$ concentrations were less than 0.02 mg/L in all conditions, there was no difference in pH from $NO_3^-$ between ABPD treatment and OBPD treatment, there was no difference between emission intensities from O radical generated by ABPD treatment and OBPD treatment, the highest $H_2O_2$ concentration was obtained by OBPD treatment, and the number of sterilized bacteria by $H_2O_2$ (5.82 mg/L) with $O_3$ (100 ppm) bubble was larger than the sum of the number of sterilized bacteria by $H_2O_2$ (5.82 mg/L) with air bubble and $O_3$ alone treatment. From these results, we conclude that the $H_2O_2$ concentration increase and the AOP by $O_3/H_2O_2$ are the decisive factor of the synergistic effect by OBPD treatment.**

***Index Terms*— Dissolved $O_3$, hydrogen peroxide, O radical, ozone bubble pulsed discharge (OBPD) treatment, sterilization effect, synergistic effect.**

## I. INTRODUCTION

INDUSTRIAL washing machines are widely used for linen service company to provide clean sheets, uniforms, and so on. In the machine, wash, rinse, and dehydration processes can be carried out with one device. In order to reduce wastewater and water consumption from the industrial washing machine, reused water that is generated after rinse and dehydration processes is purified and used to wash clothes next time. Here, if reused water is not sufficiently purified, there is a possibility that clothes and inside the industrial washing machine will be contaminated by bacteria and virus. Therefore, the reused water must be properly sterilized by dosing chemicals and/or elevating its temperature.

Recently, electric discharge is going to be applied to bactericidal inactivation in water without chemical dosing. So far, many researchers investigated the sterilization of bacteria in water by discharge inside bubble in water [1], [2], [3], underwater discharge [4], [5], and discharge on water [6]. Tanino *et al.* [1] reported that the cell density of *E. coli* suspension (*Escherichia coli* K-12, concentration: $1 \times 10^7$ viable cells/mL, and suspension volume: 2 L) decreased to four orders of magnitude within 120 s of pulsed discharge inside bubble treatment. Since the energy consumption of one-pulsed discharge and pulse repetition frequency was 0.282 J and 333 Hz, the energy efficiency for disinfection was 0.89 CFU/mL/J. Izdebski *et al.* [4] reported that concentrations of underwater microorganisms and bacteria taken from the Strzyza river in the Gdańsk region (concentration: $7.45 \times 10^4$ CFU/mL and volume: 400 mL) decreased to three orders of magnitude within 622 s by underwater spark discharge. Since pulse discharge energy and pulse repetition frequency were 0.17 J and 50 Hz, respectively, the energy efficiency was 0.19 CFU/mL/J. Hu *et al.* [6] reported that concentration of *A. baumannii* suspension (initial concentration: $1 \times 10^7$ CFU/mL) decreased to four orders of magnitude within 30 min by plasma jet on water treatment. Since plasma power was 3.8 W, the energy efficiency for disinfection was 1.46 CFU/mL/J.

Recently, we developed a continuous water treatment system consisting of an ozone generator, an ejector nozzle, and a pulsed discharge device and clarified the decomposition performance of indigo carmine solution by ozone bubble pulsed discharge (OBPD) treatment [7]. The results that we obtained were given as follows: 1) we confirmed that since the amount of O radicals and OH radicals by OBPD treatment are larger than that of by air bubble pulsed discharge (ABPD) treatment, decomposition rate of indigo carmine by OBPD treatment becomes high and 2) since the decomposition rate of OBPD treatment was higher than the sum of the decomposition rates of ABPD treatment and $O_3$ alone treatment, the synergistic effect by OBPD treatment exists. However, the sterilization effect by OBPD treatment was not clear.

In this article, we investigated the sterilization effect of simulated reused water from industrial

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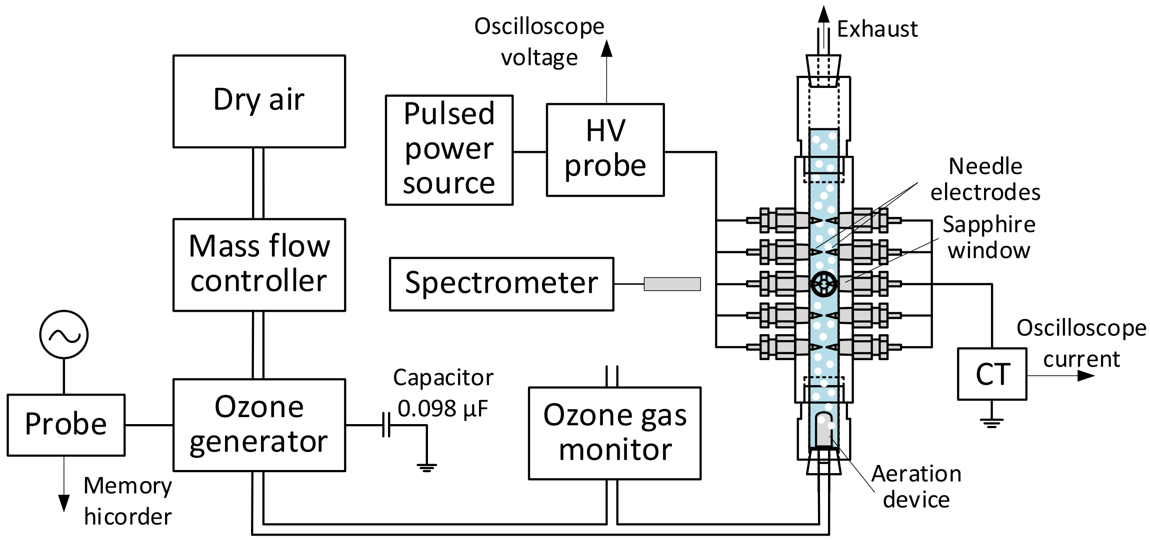



Fig. 1. Experimental setup. The system is composed of a pulse discharge device and an ozone generator.

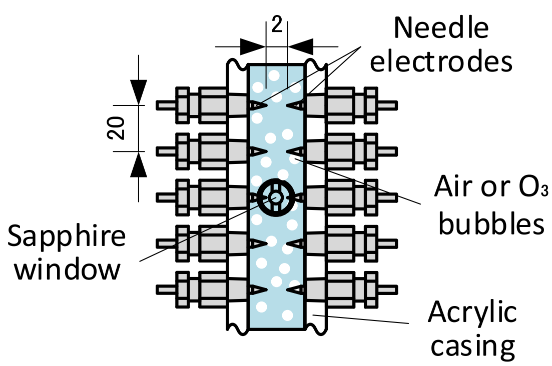



Fig. 2. Electrode system. Gap length was set to 2 mm, and needle electrodes were spaced 20 mm apart. To observe UV from pulsed discharge by the optical emission spectroscopy, the sapphire window was installed on the center part of acrylic casing.

TABLE I
EXPERIMENTAL CONDITIONS

| Parameter | Value |
|---|---|
| Volume of simulated reused water | 35 mL |
| Number of electrode pairs | 5 |
| Gap length of electrodes | 2 mm |
| Applied voltage | 30 kV |
| Pulse repetition rate | 100pps |
| Gas type | Dry air or ozone |
| Mass flow rate of gas | 0.6 L/min |
| Ozone concentration | 100ppm |
| Treatment time | 2 min and 5 min |

washing machine and sterilization mechanism by OBPD treatment.

## II. Experimental Setup

### A. Experimental Setup

Fig. 1 shows an experimental system. The system consists of a pulsed discharge device, a pulsed power supply, a mass flow controller, an ozone generator, and measurement devices.

The pulsed discharge device consists of a cylindrical acrylic vessel, five pairs of needle electrodes (curvature radius: 0.5 mm, tip angle: 15°, and material: SUS440C), an aeration device (diameter: 10 mm, type: #100, and Ibuki airstone), and sapphire window.

Fig. 2 shows the enlarged figure of the electrode system. Electrodes were spaced 20 mm apart. Each of gap lengths between needle electrodes was fixed to 2 mm. In order to observe the optical emission from electric discharges, the sapphire window was installed on the center part of the acrylic casing.

Optical emission from pulsed discharges was observed by a compact multichannel spectrometer (slit width: 200 $\mu$m, number of lines: 600 lines/mm, USB4000, and Ocean Optics). In the cases of ABPD treatment and OBPD treatment, a pulsed voltage was applied to the needle electrodes by a magnetic pulse compression-type pulsed power supply (MPC3010S-50SP, Suematsu Electronics). The applied voltage and pulse repetition rate were fixed to 30 kV and 100 pps, respectively. The applied voltage and current were measured by a high-voltage probe (PHV4002-3-R0, 100 MHz, and PMK) and a high-frequency current transformer (CT-D1.0-BNC, 500 MHz, and Magnelab), respectively. The applied voltage and the current waveform were measured by an oscilloscope (TBS2104, 100 MHz, 1 GS/s, Tektronix).

Dry air (absolute humidity: 119.3 mg/m$^3$) or ozone gas was injected into simulated reused water in the vessel via the aeration device. Its mass flow rate was fixed to 0.6 L/min by the mass flow controller (SEC400mk3, Horiba STEC). In the cases of OBPD treatment and $O_3$ alone treatment, 100 ppm of ozone was generated by the dielectric barrier discharge-type ozone generator and injected into simulated reused water in the vessel. Here, ozone gas concentration was measured by an ultraviolet absorption type ozone monitor (EG-3000B/01, Ebara Jitsugyo) and discharge power for generating ozone was set to 8.0 W.

The treatment time was set to 2 and 5 min. The sterilization experiment was carried out three times. Detailed experimental conditions are summarized in Table I.

### *B. Calculation Method for Discharge Probability, Pulsed Discharge Energy, and Pulsed Discharge Power*

In the cases of OBPD treatment and ABPD treatment, electric discharge inside bubbles dose not generated every time. Therefore, discharge probability must be considered to calculate discharge power. In this work, we calculated discharge probability by 300 times pulsed voltage application. The energy for pulsed discharge and the average power for pulsed discharge were calculated by the following equations:

$$W = \int v(t) \cdot i(t) dt \tag{1}$$

$$P = \frac{P_{\mathrm{ave}}}{100} \cdot f \cdot W. \tag{2}$$

Here, $W$ is the energy for pulsed discharge [J], $v(t)$ is the instantaneous voltage at time $t$ [V], $i(t)$ is the instantaneous current at time $t$ [A], $P$ is the average power for pulsed discharge [W], $P_{\mathrm{ave}}$ is the discharge probability [%], and $f$ is the pulse reception rate [pps].

### *C. Pulsed Discharge Observation*

A pulsed discharge appearance in simulated reused water with bubbles was observed by an ICCD camera (ULTRA-NEO, nac image technology Inc.) equipped with an UV lens (Rayfact UV-105mm F4.5, Nikon). The gate time and frame rate were fixed to 5 ns and 200 000 000 frames/s, respectively.

### *D. Preparation of Simulated Reused Water and Evaluation Method for Sterilization Effect*

To clarify the sterilization effect of reused water by OBPD treatment, a fundamental study on reused water from the washing machine installed in a linen service company was carried out.

First, we measured the viable bacteria in the reused water by colony counting. The reused water was diluted with phosphate-buffered saline by 10–10 000 times. The diluted reused water was inoculated onto agar medium, and it incubated at 35 °C for 48 h. As the result, we found that the number of viable bacteria was $1.5 \times 10^7$ CFU/mL. In this research, the initial concentration of bacterial solution was set to $1.5 \times 10^7$ count/mL.

Second, in order to decide bacterial species and genus, the reused water was analyzed by amplicon sequencing. The results were summarized in Table II. Although many kinds of bacteria were detected, we selected *Lysinibacillus odysseyi* NBRC 100172, *Aquabacterium olei* NBRC 110486, and *Acinetobacter haemolyticus* NBRC 109758 as target bacteria from the viewpoint of bio safety level 1. Since we assumed that the initial concentrations of *Lysinibacillus odysseyi*, *Aquabacterium olei*, and *Acinetobacter haemolyticus* were the same, each concentration was set to $5.0 \times 10^6$ count/mL. The initial concentration of each bacterium was controlled by using sterilized water diluted MHB liquid medium (casamino acid 17.5 mg, meat extract 3.0 mg, soluble starch 1.5 mg, and sterilized water 1000 mL). In this article, we refer to the mixed culture bacteria suspension as the simulated reused water and used it all sterilization experiments. The electrical conductivity and pH of the simulated reused water were measured by a compact conductivity meter (LAQUAtwin EC-33B, HORIBA) and a compact pH meter (LAQUAtwin B-712, HORIBA), respectively. They were 125 $\mu$S/cm and 6.7, respectively. Table III summarizes the composition of the simulated reused water.

TABLE II
RESULT OF AMPLICON SEQUENCING OF REUSED WATER TAKEN BY REAL INDUSTRIAL WASHING MACHINE

| Collection point | Taxon name | counts |
|---|---|---|
| A | *Truepera radiovictrix* | 705 |
| | *Acinetobactor dispersus* | 642 |
| | *Lysinibacillus odysseyi* | 591 |
| | *Bacillus halosaccharovorans* | 560 |
| | *Corynebacterium afermentans* | 559 |
| | Others | 4,242 |
| | Not determined | 7,610 |
| | Rejected hit | 1,737 |
| B | *Pannonibacter indicus* | 7,828 |
| | *Aquabacterium olei* | 4,238 |
| | *Novosphingobium aromaticivorans* | 897 |
| | *Cloacibacterium normanense* | 853 |
| | *Acinetobacter haemolyticus* | 348 |
| | Others | 1,150 |
| | Not determined | 572 |
| | Rejected hit | 2,223 |
| C | *Flavobacterium aquaticum* | 8,312 |
| | *Acinetobacter haemolyticus* | 2,761 |
| | *Rhizobium aggregatum* | 1,902 |
| | *Acinetobacter dispersus* | 1,276 |
| | *Pannonibacter indicus* | 1,215 |
| | Others | 2,849 |
| | Not determined | 5,112 |
| | Rejected hit | 1,233 |

TABLE III
COMPOSITION OF SIMULATED REUSED WATER

| Contents and property | | Value |
|---|---|---|
| MHB liquid medium | Casamino acid (17.5g) | 0.35 mL |
| | Meat extract (3.0 g) | |
| | Soluble starch (1.5 g) | |
| | Sterilized water (1000 mL) | |
| Sterilized water | | 34.65 mL |
| *Lysinibacillus odysseyi* | | $5.0\times10^6$ count/mL |
| *Aquabacterium olei* | | $5.0\times10^6$ count/mL |
| *Acinetobacter haemolyticus* | | $5.0\times10^6$ count/mL |
| Electrical conductivity | | 125 μS/cm |
| pH | | 6.7 |

The sterilization effect was evaluated by colony counting. The simulated reused water after treatment was diluted with phosphate-buffered saline by 10–10 000 times. The diluted simulated reused water was inoculated onto ager medium and incubated at 30 °C for 48 h.

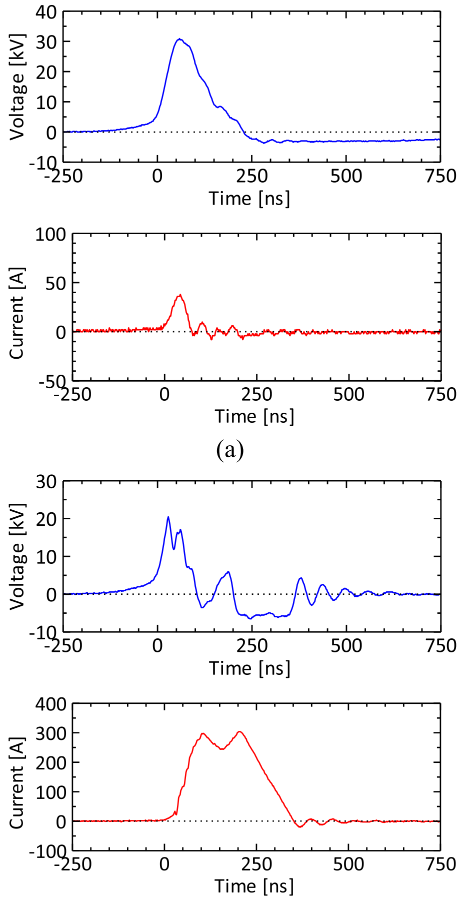



Fig. 3. Voltage and current waveforms. Applied voltage was set to 30 kV. (a) Without pulsed discharge occurrence. (b) With pulsed discharge occurrence.

The survival ratio was evaluated by the following equation:

$$\eta = \frac{N_t}{N_0}. \tag{3}$$

Here, $\eta$ is the survival ratio [-], $N_t$ is the number of viable bacteria at $t$ minute of treatment time [CFU/mL], and$N_0$ is the number of the viable bacteria at 0 min of treatment time [CFU/mL].

## III. Results

### A. Discharge Characteristics and Pulsed Discharge Appearance in the Simulated Reused Water

Fig. 3 shows the examples of voltage and current waveforms in the simulated reused water with air bubbles. From this figure, it is seen that the voltage decrease was observed after 28.4 ns of pulsed voltage application. Then, the current increased to 304 A. This tendency suggested that arc discharge generated between electrodes. Since the temperature of submerged pulsed arc plasma was around 15 000 K [8], ozone molecule in the bubble is considered to decompose by the heat from arc discharge. However, we considered that its effect is small because the discharge volume and duration of arc discharge are small.

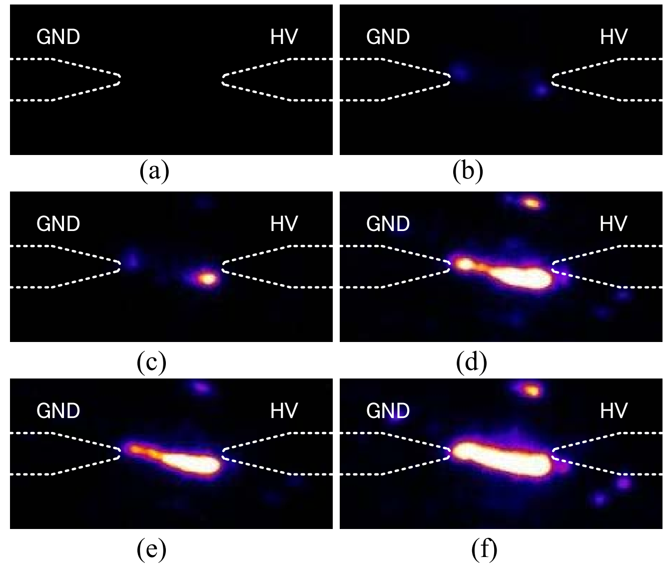



Fig. 4. Pulsed arc discharge photographs taken by the ICCD camera. The gate time and frame rate were set to 5 ns and 200 000 000 frames/s, respectively. The maximum current was 300 A. (a) $t$ ns. (b) $t + 5$ ns. (c) $t + 10$ ns. (d) $t + 15$ ns. (e) $t + 20$ ns. (f) $t + 25$ ns.

TABLE IV
Energy Efficiency for Disinfection

| Treatment methods | Energy efficiency at 5 min |
|---|---|
| ABPD | 1.83 CFU/mL/J |
| $O_3$ alone | $4.17\times10^{-4}$ CFU/mL/J |
| OBPD | 0.339 CFU/mL/J |

The energy of pulsed arc discharge for 1 discharge was 18.2 mJ. Since the discharge probability was 82.4%, the average power of pulsed arc discharge was 1.50 W. These values were the same for ABPD treatment and OBPD treatment.

Fig. 4 shows the examples of pulsed discharge photographs taken by the ICCD camera. At $t + 5$ ns [Fig. 4(b)] and $t + 10$ ns [Fig. 4(c)], partial discharges were observed at the tip of needle electrodes. After $t + 15$ ns [Fig. 4(d)–(f)], strong luminescent was observed between tips of needle electrodes. Here, strong luminescent spots were also observed in the simulated reused water region. This is due to the reflection by air bubbles.

### B. Sterilization Effect

Fig. 5 shows the sterilization effect of the simulated reused water by three kinds of treatments. Survival ratio decreased with increasing treatment time in all treatments. In the case of ABPD treatment, the survival ratio decreased by three orders of magnitude at 5 min. In the case of $O_3$ alone treatment, a survival ratio slightly decreased at 5 min. In the case of OBPD treatment, the survival ratio decreased by three orders of magnitude at 5 min. From these results, we confirmed that the highest sterilization effect was obtained by OBPD treatment.

Table IV shows the energy efficiency for disinfection by three kinds of treatments. We confirmed that the energy efficiency for disinfection by OBPD treatment was close to that reported in other plasma treatments [1], [4], [6].

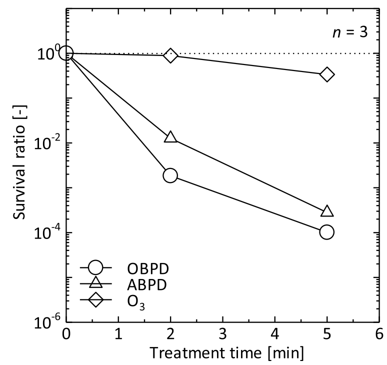



Fig. 5. Relation between survival ratio and treatment time. The discharge energy and average power of pulsed discharge were 18.2 mJ and 1.50 W, respectively. Injected ozone concentration for OBPD treatment and $O_3$ alone treatment was set to 100 ppm.

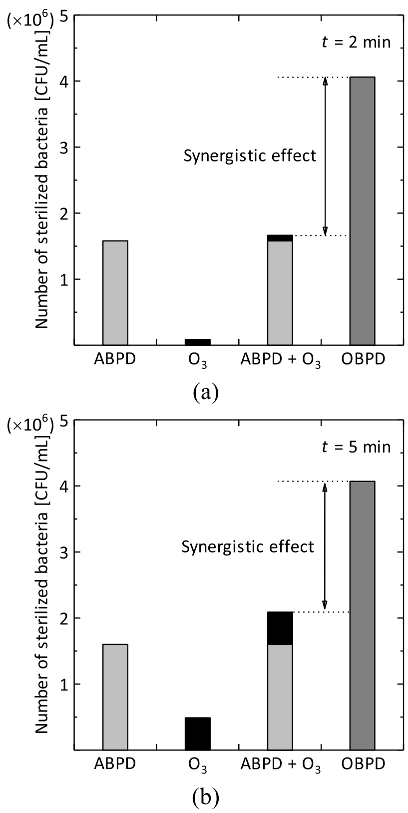



Fig. 6. Analysis of synergistic effect by OBPD treatment. ABPD + $O_3$ means the sum of the number of sterilized bacteria by ABPD treatment and that of $O_3$ alone treatment. (a) Treatment time: 2 min. (b) Treatment time: 5 min.

## *C. Synergistic Effect by OBPD Treatment*

In order to confirm the existence of synergistic effect by OBPD treatment, the results of the sterilization effects by three kinds of treatments were analyzed. If the synergistic effect by OBPD treatment exists, sterilized bacteria by OBPD treatment was higher than the sum of sterilized bacteria by ABPD treatment and $O_3$ alone treatment.

Fig. 6 shows the number of sterilized bacteria by OBPD treatment and the sum of sterilized bacteria by ABPD treatment and $O_3$ alone treatment. From Fig. 6(a), it is seen that the number of sterilized bacteria by ABPD treatment and $O_3$ alone treatment was $1.58 \times 10^6$ and $8.40 \times 10^4$ CFU/mL, respectively. Therefore, the sum of these treatments was $1.66 \times 10^6$ CFU/mL. On the other hand, the number of sterilized bacteria by OBPD treatment was $4.06 \times 10^6$ CFU/mL. Since the number of sterilized bacteria by OBPD treatment was larger than the sum of the number of sterilized bacteria by ABPD treatment and that of $O_3$ alone treatment, we confirmed that the synergistic effect for sterilization by the OBPD treatment exists. This tendency was also obtained by 5-min treatment, as shown in Fig. 6(b).

TABLE V
DISSOLVED OZONE CONCENTRATION BY THREE KINDS OF TREATMENTS

| Treatment methods | Dissolved $O_3$ concentration [mg/L] | |
|---|---|---|
| | 2 min | 5 min |
| OBPD | less than 0.02 | less than 0.02 |
| ABPD | less than 0.02 | less than 0.02 |
| $O_3$ alone | less than 0.02 | less than 0.02 |

# IV. DISCUSSION

## *A. Dissolved Ozone Concentration*

We confirmed that the sterilization effect of the simulated reused water by $O_3$ alone treatment exists. It is reported that the shock wave generated by pulsed discharge inside bubbles makes finer bubble size and larger specific area [9]. The large specific area enhances the ozone mass transfer from bubbles to the water [10]. Therefore, if the ozone gas bubble size becomes small by pulsed discharge, dissolved ozone concentration will increase and will enhance the sterilization effect by OBPD treatment. In order to confirm this hypothesis, dissolved $O_3$ concentration in the simulated reused water without bacteria was measured by a dissolved ozone concentration meter (CX-100II, Ebara Jitsugyo).

Table V shows the results of dissolved $O_3$ concentration. Although equilibrium dissolved concentration of $O_3$ was calculated to 0.05 mg/L by Henry's law in the case of $O_3$ alone treatment, dissolved $O_3$ concentrations were less than 0.02 mg/L in all conditions. This is because sufficient contact time between $O_3$ molecule in bubbles and water was not obtained. These results suggested that the dissolved $O_3$ concentration increased by pulsed discharge is not the decisive factor of the synergistic effect by OBPD treatment.

## *B. Effect of Nitrogen Oxides*

In this work, since we used dry air for generating bubble or ozone, nitrogen oxides were generated inside bubble by these plasma and chemical reactions in gas [11], [12], [13], [14]

$$e + O_2 \rightarrow e + 2O \quad (R1)$$
$$e + N_2 \rightarrow e + 2N \quad (R2)$$
$$O + O_2 + M \rightarrow O_3 + M \quad (R3)$$
$$N + O_2 \rightarrow NO + O \quad (R4)$$
$$O + NO + M \rightarrow NO_2 + M \quad (R5)$$
$$NO + O_3 \rightarrow NO_2 + O_2 \quad (R6)$$
$$NO_2 + O_3 \rightarrow NO_3 + O_2 \quad (R7)$$
$$NO + NO_2 \rightarrow N_2O_3 \quad (R8)$$

TABLE VI

$NO_3^-$ CONCENTRATION BY THREE KINDS OF TREATMENTS

| Treatment method | $NO_3^-$ concentration [ppm] | |
|---|---|---|
| | 2 min | 5 min |
| OBPD | less than 62 | less than 62 |
| ABPD | less than 62 | less than 62 |
| $O_3$ alone | less than 62 | less than 62 |

$$NO_2 + NO_3 + M \rightarrow N_2O_5 + M. \quad (R9)$$

Here, M is the third collision partner.

Since $NO_2$, $N_2O_3$, and $N_2O_5$ exist in bubbles, they transferred to water by dissolution [15], [16], [17], [18]

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3 \quad (R10)$$
$$3HNO_2 \rightarrow HNO_3 + H_2O + 2NO \quad (R11)$$
$$3NO_2 + H_2O \rightarrow 2HNO_3 + NO \quad (R12)$$
$$N_2O_3 + H_2O \rightarrow 2HNO_2 \quad (R13)$$
$$N_2O_5 + H_2O \rightarrow 2HNO_3. \quad (R14)$$

$HNO_2$ and $HNO_3$ are then ionized to $NO_2^-$ and $NO_3^-$ in the water [19], and they decrease pH of water

$$HNO_2 \rightarrow H^+ + NO_2^- \quad (R15)$$
$$HNO_3 \rightarrow H^+ + NO_3^-. \quad (R16)$$

Ikawa *et al.* [20] reported that bacteria were sterilized by plasma irradiation under low pH. Therefore, there is a possibility that the synergistic effect by OBPD treatment is due to low pH by nitric acid and nitrous acid. In order to confirm this, $NO_3^-$ and pH were measured in the simulated reused water without bacteria. Here, $NO_3^-$ concentration was measured by a compact $NO_3^-$ meter (measuring range: 62–6200 mg/L, LAQUAtwin B-743, and HORIBA).

Table VI shows the summary of $NO_3^-$ concentration measurements by three kinds of treatments. We confirmed that $NO_3^-$ concentrations were less than 62 ppm and outside of measuring range in all treatments.

Fig. 7 shows the time change of pH by three kinds of treatments. We confirmed that pH in the water decreased with increasing time in all conditions. Here, in the cases of ABPD and OBPD treatment, there was no difference on pH decrease. Therefore, we concluded that the pH decrease by nitrogen oxide is not the decisive factor of the synergistic effect by OBPD treatment.

### C. Optical Emission Spectroscopy

In the literature, emission spectra from discharge inside air bubble in water [2], [21] and underwater discharge [22], [23] were investigated by a spectrometer. They reported that the emission of the hydrogen atomic line ($H_\alpha$, $H_\beta$, and $H_\gamma$), the oxygen atomic line (O), and OH radicals was detected. These results suggested that (R1) and (R17) occur. Furthermore, in the case of discharge in $O_3$ gas and/or in $O_3$ dissolved water, (R18) and (R19) may also occur [24], [25]

$$e + H_2O \rightarrow e + OH + H \quad (R17)$$
$$e + O_3 \rightarrow e + O_2 + O + e \quad (R18)$$

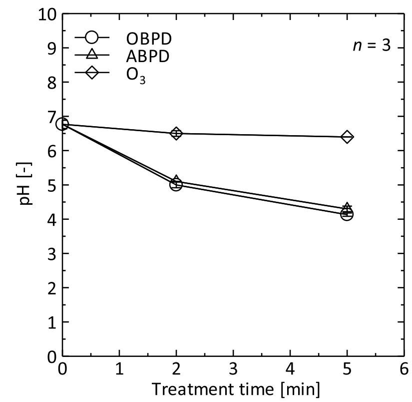
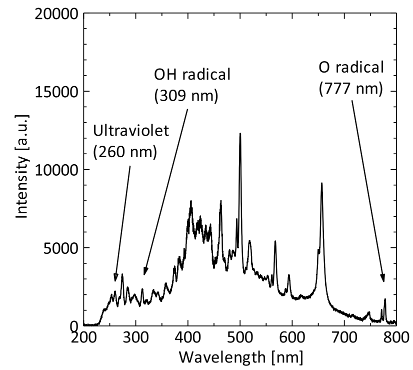
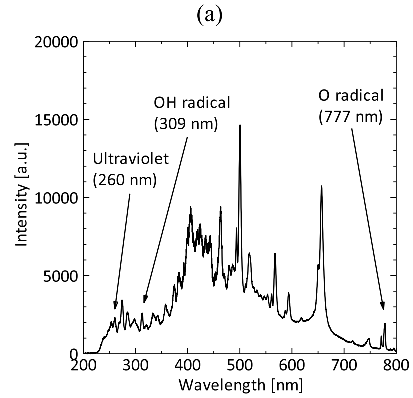



Fig. 7. Relation between pH with treatment time by ABPD treatment, $O_3$ alone treatment, and OBPD treatment. Error bar shows the standard deviation.




(a)




(b)

Fig. 8. Comparison of optical emission spectroscopy. The exposure time and the number of averaging were set to 200 ms and 20 times, respectively. (a) ABPD treatment. (b) OBPD treatment.

$$h\nu + O_3 \rightarrow O + O_2. \quad (R19)$$

Therefore, we considered that the amount of O radical generated by OBPD treatment is larger than that of by ABPD treatment. Here, it is well known that oxidation potential of O radicals (2.42 eV) is higher than $O_3$ (2.07 eV) [26]. In order to confirm this, emission spectra for OBPD treatment and ABPD treatment were observed by the spectrometer using the simulated reused water without bacteria.

Fig. 8 shows the optical emission spectra from OBPD treatment and ABPD treatment. Ultraviolet of DNA absorption wavelength (260 nm) [27], OH radicals (309 nm), and O radicals (777 nm) was detected.

Next, in order to confirm the difference between generated O radicals by OBPD treatment and ABPD treatment, the

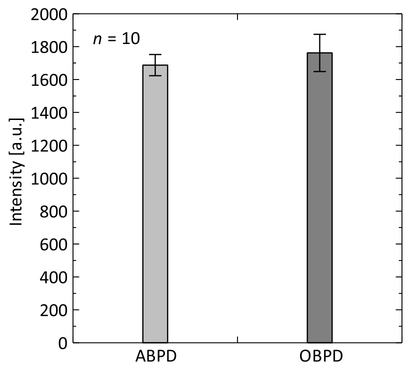



Fig. 9. Comparison of emission intensity for O radical (777 nm) by ABPD treatment and OBPD treatment. Measurement repeated by ten times. Error bar shows the standard deviation.

emission intensity for O radical was compared. Fig. 9 shows emission intensities at 777 nm by OBPD treatment and ABPD treatment. Although $O_3$ was injected into the simulated reused water, there was no difference between emission intensities of O radicals. This result suggested that O radical increase generated by pulsed discharge with $O_3$ is not the decisive factor of the synergistic effect by OBPD treatment.

### *D. Hydrogen Peroxide ($H_2O_2$) Concentration*

UV, O, and OH radicals were detected from the optical emission spectroscopy. Therefore, $H_2O_2$ is generated by these reactions [18], [28]

$$H_2O + O \rightarrow 2OH \quad (R20)$$

$$OH + OH \rightarrow H_2O_2 \quad (R21)$$

$$O_3 + h\nu + H_2O \rightarrow H_2O_2 + O_2. \quad (R22)$$

Here, $H_2O_2$ is well known for bactericides [29]. In order to confirm this, $H_2O_2$ concentration was measured by a pack test by 4-Aminoantipyline visual colorimetric method with enzyme (WAK-$H_2O_2$, Kyoritsu Chemical-Check Laboratory) and a visible spectrophotometer (ASV11D, ASONE). In this experiment, we used the simulated reused water without bacteria.

Fig. 10 shows the $H_2O_2$ concentration by three kinds of treatments. In the case of ABPD treatment, the $H_2O_2$ concentration increased with increasing treatment time. The maximum $H_2O_2$ concentration was 5.82 mg/L at 5 min. In the case of $O_3$ alone treatment, the $H_2O_2$ concentration slightly increased with increasing treatment time. The maximum $H_2O_2$ concentration was 0.12 mg/L at 5 min. In the case of OBPD treatment, $H_2O_2$ concentration increased with increasing treatment time. The maximum $H_2O_2$ concentration was 6.82 mg/L at 5 min. The highest $H_2O_2$ concentration was obtained by OBPD treatment at the same treatment time. In the case of ABPD treatment, since OH radicals were mainly generated by (R17) and (R20), $H_2O_2$ was generated by (R21). On the other hand, in the case of OBPD treatment, since injected $O_3$ reacts with UV from pulsed discharges (R22), the amount of generated $H_2O_2$ was increased in comparison with ABPD treatment. Therefore, $H_2O_2$ concentration by OBPD treatment was higher than that by the ABPD treatment. On the other hand, Ghriss *et al.* [30] reported that the reactions may occur

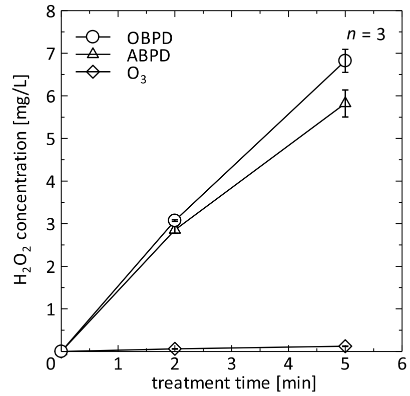



Fig. 10. Relation between $H_2O_2$ concentration and treatment time. In the cases of $O_3$ alone treatment and OBPD treatment, 100 ppm of ozone gas was injected into the simulated reused water without bacteria.

by $HNO_2$ and NO with $H_2O_2$. Since these reactions may also occur, there is a possibility that $H_2O_2$ concentration was low in the ABPD treatment

$$HNO_2 + H_2O_2 \rightarrow HNO_3 + H_2O \quad (R23)$$

$$2NO + 3H_2O_2 \rightarrow 2HNO_3 + 2H_2O_2. \quad (R24)$$

In order to clarify the mechanism of $H_2O_2$ concentration increase by ABPD treatment and OBPD treatment in detail, further study on the numerical simulation of plasma chemical reactions in water is necessary.

These results suggested that the $H_2O_2$ concentration increase is the one of the decisive factors of the synergistic effect by the OBPD treatment.

### *E. Control Experiment by $H_2O_2$ Addition With and Without $O_3$*

Since pH of the simulated reused water was 6.7, there is a possibility that advanced oxidation process (AOP) by $O_3/H_2O_2$ (R25) occurred by the OBPD treatment [31]

$$H_2O_2 + 2O_3 \rightarrow 2OH + 3O_2. \quad (R25)$$

Here, AOP is known as efficient methods to sterilize bacteria in water [31]. In order to confirm this, control experiments of $H_2O_2$ with air bubble treatment and $H_2O_2$ with 100 ppm of $O_3$ bubble treatment were conducted using the simulated reused water with bacteria. Here, initial $H_2O_2$ concentration was set to 5.82 mg/L.

Fig. 11 shows the number of sterilized bacteria by $H_2O_2$ with air bubble treatment, $O_3$ alone treatment, and $H_2O_2$ with $O_3$ bubble treatment. Here, $H_2O_2$+ $O_3$ means the sum of sterilized bacteria by $H_2O_2$ with air bubble treatment and $O_3$ alone treatment. It is seen that the number of sterilized bacteria by $H_2O_2$ with air bubble treatment and $O_3$ alone treatment was $1.20 \times 10^5$ and $4.89 \times 10^5$ CFU/mL, respectively. Therefore, the sum of these treatments was $6.09 \times 10^5$ CFU/mL. On the other hand, the number of sterilized bacteria by $H_2O_2$ with $O_3$ bubble treatment was $1.02 \times 10^6$ CFU/mL. We confirmed that the number of sterilized bacteria by $H_2O_2$ with $O_3$ bubble treatment was larger than the sum of the number of sterilized

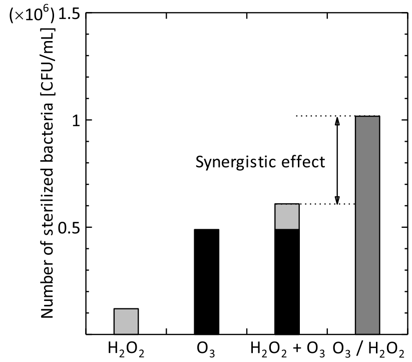



Fig. 11. Number of sterilized bacteria. Initial $H_2O_2$ and $O_3$ concentrations were set to 5.82 mg/L and 100 ppm, respectively. The treatment time was set to 5 min. Experiment repeated by three times.

bacteria by $H_2O_2$ with air bubble treatment and $O_3$ alone treatment. These results suggested that the AOP is also one of decisive factors of the synergistic effect by the OBPD treatment.

## V. Conclusion

In this article, we investigated the sterilization effect of the simulated reused water from the industrial washing machine and in order to clarify why the synergistic effect by OBPD treatment exists. The conclusions obtained are given as follows.

1) We confirmed that pulsed arc discharges are generated between tips of needle electrodes. The energy and average power for pulsed arc discharge were 18.2 mJ and 1.50 W, respectively.
2) The highest sterilization effect was obtained by the OBPD treatment.
3) Since the number of sterilized bacteria by OBPD treatment was larger than the sum of the number of sterilized bacteria by ABPD treatment and that of $O_3$ alone treatment, we confirmed that the synergistic effect by the OBPD treatment exists.
4) Dissolved $O_3$ concentrations were less than 0.02 mg/L in all conditions. Therefore, the dissolved $O_3$ concentration is not decisive factor of the synergistic effect by OBPD treatment.
5) In the cases of OBPD treatment and ABPD treatment, ultraviolet, OH radicals, and O radicals were detected by the optical emission spectroscopy. However, there was no difference on O radical intensity between ABPD treatment and OBPD treatment. Therefore, O radical increase by pulsed discharge with $O_3$ is not decisive factor of the synergistic effect by the OBPD treatment.
6) Since there was no difference in pH from $NO_3^-$ between ABPD treatment and OBPD treatment, pH decrease by pulsed discharge with $O_3$ is not decisive factor of the synergistic effect by the OBPD treatment.
7) The highest $H_2O_2$ concentration was obtained by OBPD treatment at the same treatment time. Therefore, the $H_2O_2$ concentration increase is the decisive factor of the synergistic effect by the OBPD treatment.
8) The number of sterilized bacteria by $H_2O_2$ with $O_3$ bubble treatment was larger than the sum of the number of sterilized bacteria by $H_2O_2$ with air bubble treatment and $O_3$ alone treatment. This result suggested that the AOP is also the decisive factor of the synergistic effect by the OBPD treatment.

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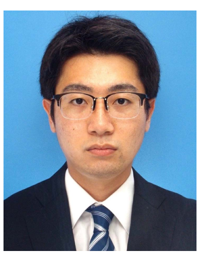

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