An Experimental Study on Disinfection System using Ozone Microbubbles Generated by the Hollow Ultrasonic Horn

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Abstract
A water disinfection treatment using ozone microbubbles generated by a hollow ultrasonic horn was developed, and its disinfection ability to Escherichia coli in water was investigated. The treatment using ozone microbubbles generated by the hollow ultrasonic horn can completely sterilize within half the time required in a treatment using bubbles generated by a disperser. These results revealed that the disinfection treatment using ozone microbubbles generated by the hollow ultrasonic horn has high mass transfer efficiency and disinfection ability.

Key words
Ozone Disinfection, Microbubble, Ultrasonic Irradiation, Radical, Escherichia coli

1. Introduction
An ozone oxidation is widely used for the water disinfection since it has powerful oxidation ability. In general, an ozone disinfection treatment is performed by bubbling an ozone gas through disperser into water [1]. However, a solubility of ozone in water is low, the ozone disinfection treatment needs long contact time or complicated contactor for an ozone dissolution into water [2].

Meanwhile, microbubbles (MB) with diameters less than 100 μm are receiving attention because of their properties such as large surface area per unit volume, low rising velocity, and self-pressurization due to surface tension. Chu et al. [1,3] reported that the ozone microbubble could make the solubility of ozone and the disinfection of microorganisms improved in comparison with conventional ozone bubble contactors at the same ozone dose. Makuta et al. [4] reported that microbubbles with diameters ranging from 5 μm to 100 μm could generate from the hollow ultrasonic horn when horn end oscillates over 10 μm. Generally, an ultrasonic oscillation is attenuated by the distance between the transducer and the bubble. This method can provide intense ultrasonic irradiation (US) close to the generated microbubbles. However, no study has attempted to enhance the microbubble generation and the ozone oxidation by ultrasonic irradiation simultaneously.

Therefore, the object of this study is to investigate and evaluate a new disinfection treatment using ozone microbubbles generated by the hollow ultrasonic horn. As a result, we revealed that this treatment has higher disinfection ability than disinfection treatments with ozone bubbling from a gas disperser and that with a strong ultrasonic irradiation.

2. Materials and Method

2.1 Bacterial preparation
A freeze-dried pellet of Escherichia coli (ATCC 8739; MicroBioLogics Inc., U.S.A.) was dissolved in 20 mL of liquid culture medium. The Escherichia coli (E. coli) solution was incubated for 20 hour in an incubator at 37°C. Test samples for disinfection were obtained by diluting 4 mL of E. coli solution to 300 mL of pure water (>18 MΩ, Direct-Q UV, Millipore Inc., U.S.A.). Test samples (~10⁸ CFU/mL) for disinfection were stored in a refrigerator at 4°C until use.

2.2 Bubble generator
Microbubbles with an average diameter less than 100 μm were generated by the hollow ultrasonic horn attached to an ultrasonic homogenizer (UH-50, SMT Co., Ltd., Japan). Fig.1 shows the photograph and the schematic diagram of the hollow ultrasonic horn. Fig.2 shows the photographs of bubbles generated by different bubble generators. The hollow ultrasonic horn was cylindrical step horn with the small section being 6 mm and the large section being 12 mm in diameter as shown in Fig.1b. There was an orifice with a diameter of 2.6 mm at the end of the small section of the horn that penetrated to the side surface of the large section. An ozone-oxygen gas mixture was supplied through a path in the hollow ultrasonic horn and produced a gas-liquid interface in water as shown in Fig.2a. When the ultrasonic horn oscillated with 40 μm peak-to-peak amplitude at 20 kHz, microbubbles were generated by the breaking up of the surface wave on the gas-liquid interface as shown in Fig.2b.

Makuta et al. [5] reported that the gas-liquid interface on the tip of needle was oscillated and microbubbles were released from the gas-liquid interface when ultrasonic irradiation. And, Brennen [6] reported that microbubbles were generated by the fission process of collapsing bubbles under the strong ultrasonic oscillation condition. Therefore microbubbles can be generated from the fragmentation of the gas-liquid interface and the fission process of bubbles by the large-amplitude oscillation of the hollow ultrasonic horn. We also used a porous gas disperser (PGD) with a pore size ranging from 40 μm to 50 μm to generate millibubbles for comparison with microbubbles as shown in Fig.2c. The diameter distributions of bubbles at a gas flow rate of 50 mL/min measured by a Laser Size Analyzer (Mastersizer 2000, Malvern Instruments Ltd., UK), we obtained that the number mean diameter of bubbles generated by the hollow ultrasonic horn and bubbles generated by the porous gas disperser were 14 and 481 μm respectively.
2.3 Experimental setup

Fig. 3 shows the schematic diagram of experimental apparatus. The test section was used a 300-milliliter glass beaker. Ozone was generated by the silent electric discharge with an ozone generator (ED-OG-R4, Ecodesign Inc., Japan) from pure oxygen gas. An ozone gas concentration and a dissolved ozone concentration in water were measured employing the flow-through ultraviolet absorption method by an ozone gas monitor (EG-600, Ebara Jitsugyo Co., Ltd., Japan) and a dissolved ozone monitor (EL-550, Ebara Jitsugyo Co., Ltd., Japan) respectively. The monitoring section of the dissolved ozone monitor cannot withstand autoclave sterilization, moreover, in case of including the impure substance like liquid culture media, the dissolved ozone concentration in water cannot be measured correctly. Therefore, dissolved ozone concentrations in pure water were measured without E. coli solution. The number of colony-forming units (CFU/mL) of E. coli was determined using a Rapid and Easy Food Bacteria Count System (DOX-30F, Bio-Theta, Ltd., Japan).

2.4 Overall mass transfer coefficient of ozone

Dissolved ozone in water self-decomposed to oxygen via complicated decomposition processes such as those represented by the SBH model [7,8] and TFG model [9]. In these models, free radicals such as superoxide radical, hydroperoxy radical, and hydroxyl radical are produced during the ozone decomposition. Chu et al. [1] reported that the ozone disinfection treatment pathways the directly attack of ozone molecule and/or indirectly attack of free radicals. Therefore, microorganisms in water are oxidized directly and/or indirectly in ozone disinfection. Especially hydroxyl radical has greater disinfection ability than ozone itself. The time derivative of the dissolved ozone concentration in the ozone dissolution model considering ozone decomposition is expressed

\[ \frac{dC}{dt} = K_La(C^* - C) - \sum_{i=1}^{m} K_{D,i} C^i \]  

where \( C \) is the dissolved ozone concentration, \( C^* \) is the saturated dissolved ozone concentration, \( K_L \) is the mass transfer coefficient, \( a \) is the total area of gas-liquid interface in unit volume, \( m \) is a fitting order, and \( K_{D,i} \) is a decomposition coefficient. In general, \( K_L \) and \( a \) are used as \( K_La \) that is called the overall mass transfer coefficient. The first term on the right-hand side is the mass flow rate of ozone dissolved from bubbles to water and the second term on the right-hand side is the mass flow rate of ozone self-decomposition in water. We estimated the overall mass transfer coefficient (\( K_La \)) from the measured value of the dissolved ozone concentration in pure water by the Boltzmann fit curve (\( m=2 \)) [2].

2.5 Disinfection kinetic model

We estimated the disinfection coefficient of ozone from obtained disinfection experimental results by using the well-known Chick-Watson model [10-13]. The Chick-Watson model is expressed.
Disinfection experiments were performed for four disinfection treatments. (1) In ultrasonic horn generated microbubble treatment (USMB treatment), a test sample is bubbled with ozone microbubbles generated by the hollow ultrasonic horn (Fig. 4a). (2) In porous gas disperser generated bubble treatment (PGD treatment), the test sample is bubbled with ozone millibubbles generated by a porous gas disperser (Fig. 4b). (3) In ultrasonic irradiation treatment (US treatment), the test sample is irradiated with an ultrasonic pressure oscillation (Fig. 4c). (4) In porous gas disperser generated bubble with ultrasonic irradiation treatment (PGD+US treatment), the test sample is bubbled with ozone millibubbles generated by a porous gas disperser and irradiated with an ultrasonic pressure oscillation located 50 mm from the disperser (Fig. 4d). Three hundred milliliters of test samples including E. coli were kept 20°C and stirred at a constant rate of 400 rpm with a cool stirrer in each treatment. The ozone gas concentration in the ozone-oxygen gas mixture was constant at 150 g/m³ and the flow rate of the ozone-oxygen gas mixture was also at 50 mL/min in USMB, PGD, and PGD+US treatments. The number of colony-forming units of E. coli was counted every 3 min for 30 min to evaluate the disinfection ability.

$$\frac{N}{N_0} = \exp(-K \bar{C} T) \quad (2)$$

where N is remaining E. coli counts, N₀ is the initial E. coli counts, T is a treating time, \(\bar{C} = \left( \int_0^T C \, dt \right) / T \) is the time-averaged ozone concentration, K is the disinfection coefficient of ozone. We used the value of the disinfection coefficient K for the qualitative evaluation of the disinfection ability.

### 2.6 Experimental procedure

Disinfection experiments were performed for four disinfection treatments. (1) In ultrasonic horn generated microbubble treatment (USMB treatment), a test sample is bubbled with ozone microbubbles generated by the hollow ultrasonic horn (Fig. 4a). (2) In porous gas disperser generated bubble treatment (PGD treatment), the test sample is bubbled with ozone millibubbles generated by a porous gas disperser (Fig. 4b). (3) In ultrasonic irradiation treatment (US treatment), the test sample is irradiated with an ultrasonic pressure oscillation (Fig. 4c). (4) In porous gas disperser generated bubble with ultrasonic irradiation treatment (PGD+US treatment), the test sample is bubbled with ozone millibubbles generated by a porous gas disperser and irradiated with an ultrasonic pressure oscillation located 50 mm from the disperser (Fig. 4d). Three hundred milliliters of test samples including E. coli were kept 20°C and stirred at a constant rate of 400 rpm with a cool stirrer in each treatment. The ozone gas concentration in the ozone-oxygen gas mixture was constant at 150 g/m³ and the flow rate of the ozone-oxygen gas mixture was also at 50 mL/min in USMB, PGD, and PGD+US treatments. The number of colony-forming units of E. coli was counted every 3 min for 30 min to evaluate the disinfection ability.

### 3. Results and Discussion

#### 3.1 Disinfection of E. coli by US, PGD, PGD+US, and USMB treatments

Fig. 5 shows the survival rates of E. coli (N/N₀) in test samples disinfected by USMB, PGD, US, and PGD+US treatments. In Fig. 5, an arrow indicates that no colony of E. coli was detected. US treatment could disinfect approximately 90% of E. coli, however could not completely sterilize the test sample within 30 min. PGD treatment could completely sterilize the sample in 30 min, and had a stronger disinfection effect than US treatment. PGD treatment employs the chemical oxidation of ozone to kill E. coli; meanwhile, US treatment uses physical pressure to kill. Therefore, these results reveal that US treatment is difficult to kill E. coli far from the horn and attached to the wall of the beaker, and PGD treatment can effectively sterilize when the dissolved ozone concentration is maintained over the threshold value.

USMB treatment could completely sterilize the sample in 15 min, which is only half the time required in PGD treatment. USMB treatment had a much stronger disinfection effect than PGD treatment. Meanwhile, PGD+US treatment, in which the bubble generator and the ultrasonic device are separately located, could completely sterilize the sample in 27 min. This result reveals that the synergistic effect of physical and chemical disinfection effect by USMB treatment, can effectively sterilize than PGD+US treatment, in which an ultrasonic pressure from the ultrasonic device is attenuated near the bubble generator.

#### 3.2 Effect of the dissolved ozone concentration on the E. coli disinfection

The disinfection ability of ozone treatment is generally proportional to the CT value, which is the product of the time-averaged dissolved ozone concentration (\(\bar{C}\)) and treating time (T). Therefore, we measured the dissolved ozone concentration in cases of bubbles generated from the hollow ultrasonic horn, the disperser with and without ultrasonic irradiation. Fig. 6 shows the time evolution of dissolved ozone concentration for the test samples employing USMB, PGD, and PGD+US treatments. The high dissolution rate of ozone microbubbles generated by hollow ultrasonic horn made increased the dissolved ozone concentration quickly. The overall mass transfer coefficients (K₀,a) of ozone in the cases of bubbles generated from the hollow ultrasonic horn, the disperser with and without ultrasonic irradiation were 0.073, 0.032 and 0.040 min⁻¹ respectively. Therefore, the high dissolution ability of microbubbles increased the dissolved ozone concentration quickly and enhanced the disinfection ability of USMB treatment.

Fig. 7 shows the disinfection curves for 99.99% E. coli disinfection (N/N₀ > 10⁶) estimated by the Chick-Watson model (Eq. (2)) with experimental data of disinfection rate and dissolved ozone concentration. All the correlation coefficients (R) between experimental results and the Chick-Watson model were sufficiently high (R > 0.97).

Table 1 shows the disinfection coefficients (K) in USMB, PGD, and PGD+US treatments estimated by the
Chick-Watson model. USMB treatment was the most powerful disinfection treatment of three treatments. USMB and PGD+US treatments were 3.11 and 1.36 times more effective than PGD treatment. Especially, although PGD+US treatment was low dissolved ozone concentration than PGD treatment, it had a higher disinfection coefficient than PGD treatment. These results reveal that the synergistic effect of physical effect for ultrasonic irradiation and chemical oxidation for ozone can enhance the disinfection ability for E. coli.

Meanwhile, the calculation for the disinfection coefficients used the dissolved ozone concentration in pure water instead of the immeasurable that in test sample. The mass transfer coefficient (\(K_L\)) of ozone in pure water seems to be approximately same among USMB, PGD, and PGD+US treatments in spite of varying in surface area (a) and it is generally higher than that in including impurities that interfere the smooth mass transfer. Fig.8 shows the calculated values of \(K\) in cases of tenth, hundredth and thousandth of \(K_L\) in pure water. As shown in Fig.8, the trend of disinfection abilities among USMB, PGD, and PGD+US treatments were consistent, e.g., values of \(K\) in USMB and PGD+US treatments in cases of thousandth of \(K_L\) in pure water were 4.42 and 1.74 times higher than PGD treatment respectively. Therefore, those in actual test sample were considered to be consistent qualitatively.

### 3.3 Effect of ultrasonic irradiation on the E. coli disinfection

The physical disinfection effect of ultrasonic irradiation only for E. coli is evidently low in comparison with ozone disinfection treatments such as USMB, PGD, and PGD+US treatments. However, in ozone disinfection, there are generally direct attack of ozone molecule and indirect attack of free radicals, which produced during the decomposition from ozone to oxygen [1]. Ince et al. [14] reported that ultrasonic irradiation enhances ozone decomposition and produces hydroxyl radicals, which have great disinfection ability. In general, a dissolved ozone is decomposed in water and the dissolved ozone concentration gradually decreases. Fig.9 shows the time evolution of the dissolved ozone concentration in pure water when the initial dissolved ozone concentration was 14.5 mg/L and test samples were stirred at 400 rpm without an ozone gas supply. The dissolved ozone concentration in the case with ultrasonic irradiation clearly decreased more quickly than that in the case without ultrasonic irradiation. This result reveals that ultrasonic irradiation enhances the ozone decomposition. Moreover, this result can also explain that the time evolution curve of the dissolved ozone concentration of PGD+US treatment as shown in Fig.6 is clearly below than that of PGD treatment in spite of the same ozone supply condition. Therefore, these results reveal that ultrasonic irradiation enhances self-decomposition of ozone in addition to ultrasonic physical disinfection, and it makes the disinfection of USMB and PGD+US treatments more effective.
Fig. 8 Calculated values of $K$ in cases of tenth, hundredth and thousandth of $K_L$ in pure water in USMB, PGD, and PGD+US treatments.

Fig. 9 Decomposition curves of ozone with/without ultrasonic irradiation when the initial dissolved ozone concentration was set to 14.5 mg/L without the supply ozone.

4. Conclusion
We developed a new disinfection treatment that uses ozone microbubbles generated by the hollow ultrasonic horn (USMB treatment) and investigated its disinfection ability. USMB treatment has higher disinfection ability than conventional treatments such as treatment with ozone bubbles generated by the porous gas disperser (PGD treatment), treatment with ultrasonic irradiation to test sample (US treatment), and treatment with ozone bubbles generated by the porous gas disperser and ultrasonic irradiation (PGD+US treatment). Especially, the disinfection coefficient ($K$) of USMB treatment is more than three times higher than that of PGD treatment. The overall mass transfer coefficient of ozone ($K_{L_a}$) of USMB treatment is $0.073 \text{ min}^{-1}$ and approximately twice that of PGD and PGD+US treatments. In addition, the dissolved ozone with ultrasonic irradiation decomposed more quickly than that without ultrasonic irradiation and generated free radicals that have high disinfection ability. Consequently, these results reveal that the effect of ultrasonic irradiation enhances self-decomposition of ozone in addition to ultrasonic physical disinfection, and it makes the disinfection of USMB and PGD+US treatments more effective.

Nomenclature
- $C$: dissolved ozone concentration, mg/L
- $C^*$: saturated dissolved ozone concentration, mg/L
- $K_L$: mass transfer coefficient of ozone, m/min
- $a$: total area of gas-liquid interface in unit volume, m$^{-1}$
- $K_{L_a}$: overall mass transfer coefficient of ozone, min$^{-1}$
- $m$: fitting order
- $K_D$: decomposition coefficient of ozone, min$^{-1}$
- $T$: treating time, min
- $N$: remaining E. coli counts, CFU/mL
- $N_0$: initial E. coli counts, CFU/mL
- $\overline{C}$: time-averaged ozone concentration, mg/L
- $K$: disinfection coefficient of ozone, L/(mg·min)
- $R$: correlation coefficient

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References


