PAPER

Inactivation of *Escherichia coli*, *Saccharomyces cerevisiae* and *Bacillus subtilis* by ultrasonic cavitation

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Abstract: In recent years, inactivation (sterilization) of bacteria using ultrasonic waves has attracted significant attention. However, the details of the inactivation mechanisms have not been elucidated. This study aims to clarify the inactivation mechanisms of bacteria and fungi by ultrasonic cavitation. *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*) were used as the test bacteria, and *Saccharomyces cerevisiae* (*S. cerevisiae*) as the test fungi. Inactivation was attempted by ultrasonic irradiation at frequencies of 20 kHz to 4.4 MHz and an acoustic power density of 0.1 W/mL. Different frequency dependences of the inactivation were confirmed in *E. coli*, *S. cerevisiae*, and *B. subtilis*. The highest inactivation rate in *E. coli* was observed at 430 kHz. The effect of ultrasonic cavitation on *E. coli* was examined. We investigated the inactivation rate of *E. coli* when the sonochemical efficiency was kept constant at different frequencies (200, 430, and 950 kHz) by adjusting the acoustic power. The inactivation rates at different frequencies showed a similar time dependence. In contrast, *B. subtilis* spores were inactivated after increasing the power density. Based on these data, the mechanism of inactivation is discussed with a focus on cell characteristics.

Keywords: Ultrasonic cavitation, *E. coli*, *S. cerevisiae*, *B. subtilis*, OH radical

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1. INTRODUCTION

Methods for inactivating bacteria include the use of heat, ultraviolet light, and chemicals [1]. Among these, chlorination is popular because it is inexpensive and simple. However, chlorination involves issues such as damage to the human body, environmental pollution, and equipment damage due to corrosion. In contrast, inactivation of bacteria using ultrasonic waves is not associated with such issues. The negative pressure due to the application of sonication to the solute results in the generation of bubbles. This phenomenon is called ultrasonic cavitation [2–7]. The cause cavitation bubbles repeatedly expand and contract [3], and generate physical effects such as shock waves, shear stress, and micro-jets. The collapse of bubbles results in high temperature and pressure fields, i.e., “hot spots,” through adiabatic compression. The hot spots result in chemical effects such as the generation of OH radicals and hydrogen peroxide

through thermal decomposition. Previous studies [8–15] have reported that these effects lead to the inactivation of microorganisms. Duan *et al.* [16] and Kurokawa *et al.* [17] destroyed algae using ultrasonic treatment. Kang *et al.* [18] and Loredó *et al.* [19] achieved ultrasonic inactivation of bacteria in food. Gao *et al.* [20] reported a decrease of approximately 4.5 logs when *B. subtilis* was irradiated by 20 kHz ultrasonic waves for 20 min. In addition, Koda *et al.* [21] and Hua *et al.* [22] reported that OH radical production by ultrasonic treatment at several hundred kilohertz (kHz) affected bacterial inactivation. Guerrero *et al.* [23] reported that the inactivation mechanism was damage to yeast cells by ultrasonic cavitation. Thus, the inactivation mechanism of inactivation differs depending on various parameters such as the ultrasonication frequency and acoustic power, and the structure of the microorganisms. In particular, bacteria are divided into gram-positive and gram-negative types [24–27]. Ultrasonic inactivation is thought to depend on the structure of the microbial cells [28,29]. The effect of ultrasonic cavitation on the surroundings is considered to be a factor in the inactivation of bacteria and fungi; however, this has not been clearly

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determined. Our research thus far has been aimed at clarifying the destruction mechanism of algae by irradiating ultrasonic waves with different frequencies and constant acoustic power [17,30]. The purpose of this study is to elucidate the mechanism of bacteria and fungi inactivation by ultrasonic treatment. We investigated the effects of ultrasonic treatment on *Escherichia coli* (*E. coli*), *Saccharomyces cerevisiae* (*S. cerevisiae*), and *Bacillus subtilis* (*B. subtilis*). The experiment was conducted at frequencies from 20 kHz to 4.5 MHz and an acoustic power of 10 W. The dependence of inactivation of *E. coli* on acoustic power was also examined.

2. MATERIALS AND METHODS

2.1. Sample Preparation

The following strains, supplied by National Institute of Technology and Evaluation Biological Resource Center, (NBRC, Japan), were used in this study: *E. coli* NBRC 108669, *S. cerevisiae* NBRC 1346, and *B. subtilis* NBRC 3134. Table 1 shows the characteristics of the cells. *E. coli* was grown in L medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) for 4 h at 30°C. *S. cerevisiae* was grown in YM medium (10 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, and 3 g/L malt extract) for 5 h at 30°C. *B. subtilis* (vegetative cell) was grown in 802 medium (10 g/L peptone, 2 g/L yeast extract, 1 g/L MgSO₄·7H₂O) for 4 h at 30°C. In addition, *B. subtilis* spores were grown for 7 days (at 30°C, 50% relative humidity) on 802 agar medium at a concentration of 10%. After incubation, the cells collected by centrifugation were suspended in sterile ultrapure water. The sample concentrations were adjusted using a spectrophotometer (UV-1800, Shimadzu Corporation, Japan). The initial colony-forming unit (CFU) numbers were 10⁷ CFU/mL for *E. coli*, 10⁶ CFU/mL for *S. cerevisiae*, and 10⁴ CFU/mL for *B. subtilis*.

2.2. Experimental Procedures

Ultrasonic irradiation was performed using four types of reactors. Figures 1 and 2 depict the schematics of two of the reactors used in experiments. The reactor shown in Fig. 1 was used to irradiate *E. coli* and *S. cerevisiae*. Ultrasound was produced from an ultrasonic generator (QUAVA mini, Kaijo, Japan) in a stainless steel cylindrical bath installed on the transducer. The frequencies used

Fig. 1 Schematic diagram of the sonochemical reactor (QUAVA mini) for the ultrasonic irradiation of *E. coli* and *S. cerevisiae* suspensions at 26 kHz, 200 kHz, 430 kHz, 950 kHz, 1.6 MHz, and 3.6 MHz.

Fig. 2 Schematic diagram of the sonochemical reactor for ultrasonic irradiation of *B. subtilis* suspension at 20 kHz (a), and 0.4, 1.0, 2.3, 3.4, and 4.4 MHz (b).

Fig. 3 Schematic diagram of the sonochemical reactor (QUAVA mini) for ultrasonic irradiation of *B. subtilis* spore suspension at 1.6 MHz.

Table 1 Cellular characteristics of bacteria and fungi.

cell	taxon	size	shape	Gram stain
<i>E. coli</i>	bacterium	3.1 × 1.0 μm	rod	Gram-negative
<i>S. cerevisiae</i>	fungus	4.6 μm (diameter)	coccus	—
<i>B. subtilis</i> (vegetative cell)	bacterium	7.8 × 1.1 μm	rod	Gram-positive
<i>B. subtilis</i> (spore)	bacterium	1.5 × 0.7 μm	rod	Gram-positive

were 26, 200, 430, and 950 kHz, and 1.6 and 3.6 MHz. Figure 2 shows the reactor used for the ultrasonic irradiation of *B. subtilis*. The piezoelectric lead zirconate titanate disk-type vibrator (Fuji Ceramics, Japan) is 30 mm in diameter and set at the bottom of a stainless steel cylinder. The frequencies used for this setup were 400 kHz, and 1.0, 2.3, 3.4, and 4.4 MHz. A horn-type ultrasonic homogenizer (VC750, SONIC & MATERIALS, INC.) of 13 mm diameter was used for the low frequency of 20 kHz. The setup in Fig. 3 was used to inactivate *B. subtilis* spores. It has two vibrators generating a frequency of 1.6 MHz. The suspension (100 mL) was placed in a stainless steel cylinder and sonicated for 30 min. The acoustic power was regulated to 10 ± 1 W by calorimetry [29,31,32]. The sample temperature was regulated at 20°C to prevent thermal inactivation. All experiments were performed in triplicate, and the presented results are the average inactivation rates.

2.3. Analytical Methods

2.3.1. Calculation of inactivation rate

Samples were taken before and after sonication. The number of bacteria or fungi was determined by colony count after incubating the samples at 37°C for 24 h. The inactivation rate was calculated using the following Eq. (1):

$$[\%] = 100 - \left(\frac{N}{N_0} \right) \times 100 \quad (1)$$

where N and N_0 are the counts of bacteria or fungi before and after inactivation, respectively.

2.3.2. Particle size analysis and scanning electron microscopy (SEM)

The particle size was evaluated using a laser diffraction particle size analyzer (SALD-2300, Shimadzu Corporation, Japan). The measurement was performed on the solution obtained by diluting the sample three times in the case of *S. cerevisiae*. The SEM investigation was performed using a TM3030Plus SEM (Hitachi High-Tech Corporation, Japan). The volume ratio of the sample to the staining solution was 5:1.

2.3.3. Turbidity

Turbidity was measured using an ultraviolet–visible spectrophotometer (UV-1800, Shimadzu Corporation, Japan) at OD₆₅₀ (optical density at 650 nm wavelength).

2.4. Determination of Chemical Effect

The KI method [32,33] was used to quantify the chemical effects of ultrasonic cavitation. I^- ions are oxidized by chemical species such as OH radicals and hydrogen peroxide generated by cavitation bubbles. I_2 has poor solubility in water and reacts with I^- to form I_3^- , which has a characteristic absorbance peak at 355 nm. The amount of I_3^- produced was estimated from this absorb-

ance. In addition, the amount of chemical species generated per unit of ultrasonic energy [mol/J] (sonochemical efficiency, hereinafter referred to as *SE*) was calculated from the absorbance. The *SE* is calculated using the following Eq. (2):

$$SE = \frac{cV}{Pt} \quad (2)$$

where c [mol/dm³] is the molarity of I_3^- ions generated from 0.1 mol/dm³ KI solution, V [dm³] is the volume of KI solution, P [W] is the acoustic power, and t [s] is the ultrasonic irradiation time.

3. RESULTS AND DISCUSSION

3.1. *E. coli*

3.1.1. Inactivation rate

Figure 4 shows the inactivation rate of *E. coli* versus irradiation time at 26 kHz to 3.6 MHz using the reactor shown in Fig. 1. At frequencies of 430 kHz and 950 kHz, the irradiation times for obtaining an inactivation rate of approximately 100% were 10 min and 25 min, respectively. Conversely, at frequencies of 26 kHz and 3.6 MHz, the inactivation rate was 20% after 30 min of irradiation. Although not completely inactivated, the inactivation rate was 80% at 1.6 MHz. Figure 5 shows the inactivation rate of *E. coli* after sonication for 10 min. The frequency with the highest inactivation rate was 430 kHz. Low inactivation rates were obtained at 26 kHz and 3.6 MHz. These results confirm the frequency dependence of inactivation.

Koda *et al.* [21] reported that ultrasonic inactivation of *E. coli* is slightly more effective at 500 kHz than at 20 kHz. In previous studies [8,21,22], horn-type transducers were used for frequencies in the 20 kHz band. The horn-type transducer has a larger vibration displacement than the disk-type transducer and hence, a larger local energy density. Therefore, it was difficult to compare the results obtained from a horn-type transducer with those obtained

Fig. 4 Inactivation rate of *E. coli* against irradiation time at acoustic power of 10 W: ◆ 26 kHz, ■ 430 kHz, ▲ 950 kHz, × 1.6 MHz, and ○ 3.6 MHz.

Fig. 5 Frequency dependence of *E. coli* inactivation rate for 10 min irradiation at an acoustic power of 10 W.

Fig. 6 Frequency dependence of sonochemical efficiency at an acoustic power of 10 W.

Fig. 7 Inactivation rate of *E. coli* against irradiation time at constant reaction rate: — 200 kHz, × 430 kHz, and ▲ 950 kHz.

Fig. 8 Acoustic power dependence of *E. coli* inactivation for 5 min irradiation.

using a high-frequency disk-type transducer. In this study, the same reactor was used from 26 kHz to 3.6 MHz. Hence, we were able to maintain the same acoustic intensity across a broad band of frequencies.

3.1.2. Effect of chemical action

The collapse of cavitation bubbles causes chemical action by OH radicals and physical action such as shear stress. The chemical action can be quantified by the KI method. Figure 6 illustrates the calculated *SE* at an acoustic power of 10 W using the KI method. The *SE* was highest at 430 kHz, and lowest at 26 kHz and 3.6 MHz. The *SE* and inactivation rate of *E. coli* in Figs. 5 and 6 had the same frequency dependence. We hypothesized that the inactivation rate would be the same if the experiment was conducted with the same *SE* at different frequencies. The amount of chemical species (reaction rate) [34] generated per unit time was maintained at a constant value by changing the acoustic power while setting the frequency at 200 kHz, 430 kHz, and 950 kHz. Figure 7 shows the inactivation rate of *E. coli* at a constant reaction rate of 5.9 ± 0.3 [10⁻⁹ mol/s]. The inactivation rates at different frequencies showed a similar time variation. Thus, the inactivation of *E. coli* depends on the reaction rate. This

suggests that the main factor in the inactivation mechanism of *E. coli* is the chemical action of ultrasonic cavitation.

3.1.3. Dependence on acoustic power

The dependence of inactivation on the acoustic power was investigated at 430 kHz, which, as aforementioned, is the most effective frequency for deactivation. The inactivation rates of irradiation for 5 min at varying acoustic powers is shown in Fig. 8. At the acoustic powers of 10, 20, and 30 W, the inactivation rate increased as the power increased. However, at 40 and 50 W, the inactivation rate decreased relative to that at 30 W. This reduction is because of the saturation and reduction of chemical action with increasing acoustic power density.

3.2. *S. cerevisiae*

3.2.1. Inactivation rate

Figure 9 shows a graph of the time dependence of the inactivation rate of *S. cerevisiae* at each frequency. The irradiation reactor for *S. cerevisiae* is shown in Fig. 2. At the frequencies of 950 kHz and 1.6 MHz, the inactivation rate was approximately 100% for 5 min irradiation. *S. cerevisiae* was not inactivated at the low frequencies of 26 kHz and 430 kHz. The frequency dependence of the

Fig. 9 Inactivation rate of *S. cerevisiae* against irradiation time at an ultrasonic power of 10 W: ◆ 26 kHz, ■ 430 kHz, ▲ 950 kHz, × 1.6 MHz, and + 3.6 MHz.

Fig. 10 Frequency dependence of *S. cerevisiae* inactivation rate for 5 min irradiation at an ultrasonic power of 10 W.

S. cerevisiae inactivation rate for 5 min irradiation is shown in Fig. 10. The effective frequencies for inactivation were 950 kHz and 1.6 MHz. Thus, the frequency character-

istics of the inactivation rates differ between *E. coli* and *S. cerevisiae*.

3.2.2. Particle size distribution

Changes in the particle size of *S. cerevisiae* by ultrasonic treatment was investigated. Figure 11 shows the particle size of the cells before and after ultrasonic irradiation. Each cell particle was 5 μm in diameter before irradiation. The cell size was significantly changed by ultrasonic irradiation at 950 kHz and 1.6 MHz. This indicates that the cells were broken. No changes in the particle sizes could be detected at 3.6 MHz, at which the inactivation rate was 60%. After ultrasonic irradiation for 30 min at a frequency of 430 kHz, the diameter increased by 2–4 μm . In Fig. 9, a negative inactivation rate was obtained at 430 kHz. The negative inactivation rate is attributed to cell agglutination during the budding process. As cell aggregation occurs when daughter cells budding from mother cells do not divide completely, we speculate that these cells were split apart by ultrasonic cavitation.

3.2.3. Mechanical resonance

The results of Sects. 3.2.1 and 3.2.2 suggest that inactivation of *S. cerevisiae* is due to the mechanical effects of cavitation bubbles. We investigated the mechanical resonance of the cell walls, since it was confirmed that there is a frequency dependence of inactivation has been confirmed. A shell model [35] was used to analyze the resonance frequency of microorganisms. The resonance frequency of *S. cerevisiae* can be calculated from Eq. (3):

$$f_K = \frac{1}{2\pi} \sqrt{\frac{Eh}{2\rho a^3(1-\nu)}} \quad (3)$$

where f_K is the resonance frequency [Hz], E is the Young's modulus [N/m^2], h is the cell thickness [m], ρ is the cell density [kg/m^3], a is the cell radius [m], and ν is the

Fig. 11 Particle size distribution of *S. cerevisiae* before and after sonication for 30 min at 26, 430, 950 kHz, and 1.6 and 3.6 MHz: ◆ before, × after.

Table 2 Resonance frequency calculated from Young's modulus measured by SPM.

Young modulus	Resonance frequency
10–23.0 MPa	0.7–1.1 MHz

Poisson's ratio. The Young's modulus was measured using a scanning probe microscope (SPM-9700, Shimadzu Corporation). Table 2 shows the measured Young's modulus and the calculated resonance frequency.

The frequencies with the highest inactivation rates were 950 kHz and 1.6 MHz, which are comparable to the resonance frequency. In addition, Table 3 shows the resonant bubble diameter. The bubble size at the frequency effective for inactivation is the same as the cell size. Thus, one of the inactivation mechanisms for *S. cerevisiae* is cell wall mechanical resonance.

3.3. *B. subtilis*

3.3.1. Inactivation rate

Figure 12 shows the inactivation rate when *B. subtilis* vegetative cells and spores were sonicated for 15 min. The reactor shown in Fig. 2 was used for *B. subtilis*. The horn-type vibrator produced ultrasound of only 20 kHz. Vegetative cells showed high inactivation rates at 20 kHz and 410 kHz. Frequencies in the (megahertz) MHz band were unsuitable for inactivation. Cells irradiated with 20 kHz ultrasound for 30 min were observed with a scanning electron microscope (TM3030Plus, Hitachi). As shown in Fig. 13, there were cells that ruptured in the minor axis direction. Therefore, the inactivation of *B. subtilis* vegetative cells is considered to be through physical cell destruction by ultrasonic cavitation. In contrast, the spores were not inactivated at any frequency.

Fig. 12 Frequency dependence of *B. subtilis* vegetative cell and spore inactivation rate for 15 min irradiation at ultrasonic power of 10 W: □ vegetative cell and ◇ spore.

3.3.2. Inactivation of spores by high power density

Spores are covered by hard shells and are highly resistant to heat and chemicals. To increase the power density, the reactor was changed to that shown in Fig. 3. We tried sonication with acoustic power densities of 1.24 W/mL and 2.49 W/mL at 1.6 MHz. The results are shown in Fig. 14. In the case of 1.24 W/mL, the spore inactivation rate was 75% after 60 min. When the acoustic power density increased, more spores were inactivated at the same irradiation time.

3.3.3. Relationship between inactivation rate and turbidity

Previous studies have reported that turbidity decreases when *B. subtilis* spores are inactivated [36,37]. Figure 15 shows the inactivation rate of spores and the optical density at 650 nm when the acoustic power density was 1.24 W/mL. As the inactivation rate increased, OD₆₅₀ decreased. Thus, *B. subtilis* spores could be inactivated by

Table 3 Resonant bubble radius at ultrasonic frequency.

Frequency	26 kHz	430 kHz	950 kHz	1.6 MHz	3.6 MHz
Equilibrium radius [μm]	164	8.7	3.7	1.8	1.3

Fig. 13 SEM images of *B. subtilis* vegetative cells before (a) and after ultrasound irradiation for 30 min (b), (c).

Fig. 14 Inactivation rate of *B. subtilis* spore against irradiation time at the ultrasonic powers of ○ 1.24 W/mL and △ 2.49 W/mL.

Fig. 15 Irradiation time variation of inactivation rate at 1.6 MHz and 1.24 W/mL, and optical density (OD₆₅₀) of *B. subtilis* spore.

ultrasound by changing the power density from 0.1 to 1.24 W/mL.

4. CONCLUSION

Inactivation of bacteria and fungi by ultrasonic treatment was investigated using *E. coli*, *S. cerevisiae*, and *B. subtilis*. The frequency dependence of the inactivation rate was different for each bacterium and fungus. This result suggests a difference in the inactivation mechanism depending on the microorganism. For *E. coli*, 430 kHz was the most effective frequency for inactivation. It was speculated that OH radicals and H₂O₂ were involved. *S. cerevisiae* was inactivated at frequencies of 950 kHz and 1.6 MHz. Based on the analysis results using a shell model, the inactivation of *S. cerevisiae* is thought to be owing to physical destruction of the cells. *B. subtilis* vegetative cells showed high inactivation rates at 20 kHz and 410 kHz. SEM images confirmed cell destruction. The inactivation of *B. subtilis* vegetative cells is because of the physical destruction of the cells. In contrast, *B. subtilis* spores were not inactivated at 0.1 W/mL. However, they were successfully inactivated by increasing the power density. These

results indicate that the inactivation mechanism varies depending on the characteristics of the cells.

REFERENCES

- [1] S. Isobe, "Advanced micro-organism control system with non-thermal processing," *J. Food Microbiol.*, **27**, 115–122 (2010) (in Japanese).
- [2] T. G. Leighton, *The Acoustic Bubble* (Academic Press, London, 1994).
- [3] K. Yasui, "Influence of ultrasonic frequency on multibubble sonoluminescence," *J. Acoust. Soc. Am.*, **112**, 1405 (2002).
- [4] K. Yasuda, "Application of sonochemistry to treatment of water pollutant," *Netsu Sokutei*, **40**, 58–64 (2013) (in Japanese).
- [5] B. Verhaagen and D. F. Rivas, "Measuring cavitation and its cleaning effect," *Ultrason. Sonochem.*, **29**, 619–628 (2016).
- [6] W. B. McNamara, Y. T. Didenko and K. S. Suslick, "Pressure during sonoluminescence," *J. Phys. Chem. B*, **107**, 7303 (2003).
- [7] K. Yasui, T. Tuziuti and Y. Iida, "Dependence of the characteristics of bubbles on types of sonochemical reactors," *Ultrason. Sonochem.*, **12**, 43–51 (2005).
- [8] M. Furuta, M. Yamaguchi, T. Tsukamoto, B. Yim, C. E. Stavarache, K. Hasiba and Y. Maeda, "Inactivation of *Escherichia coli* by ultrasonic irradiation," *Ultrason. Sonochem.*, **11**, 57–60 (2004).
- [9] J. Raso, R. Pagan, S. Condon and F. J. Sala, "Influence of temperature and pressure on the lethality of ultrasound," *Appl. Environ. Microbiol.*, **64**, 465–471 (1998).
- [10] M. M. Rahman, K. Ninomiya, C. Ogino and N. Shimizu, "Ultrasound-induced membrane lipid peroxidation and cell damage of *Escherichia coli* in the presence of non-woven TiO₂ fabrics," *Ultrason. Sonochem.*, **17**, 738–743 (2010).
- [11] G. Hunter, M. Lucas, I. Watson and R. Parton, "A radial mode ultrasonic horn for the inactivation of *Escherichia coli* K12," *Ultrason. Sonochem.*, **15**, 101–109 (2008).
- [12] D. Liu, X.-A. Zeng, D.-W. Sun and Z. Han, "Disruption and protein release by ultrasonication of yeast cells," *Innov. Food Sci. Emerg. Technol.*, **18**, 132–137 (2013).
- [13] C. Dai, F. Xiong, R. He, W. Zhang and H. Ma, "Effects of low-intensity ultrasound on the growth, cell membrane permeability and ethanol tolerance of *Saccharomyces cerevisiae*," *Ultrason. Sonochem.*, **36**, 191–197 (2017).
- [14] Y. Iida, T. Tuziuti, K. Yasui, T. Kozuka and A. Towata, "Protein release from yeast cells as an evaluation method of physical effects in ultrasonic field," *Ultrason. Sonochem.*, **15**, 995–1000 (2008).
- [15] G. Scherba, R. M. Weigel and W. D. O'Brien, Jr., "Quantitative assessment of the germicidal efficacy of ultrasonic energy," *Appl. Environ. Microbiol.*, **57**, 2079–2084 (1991).
- [16] Z. Duan, X. Tan, K. Dai, H. Gu and H. Yang, "Evaluation on H₂O₂-aided ultrasonic pretreatment for cell disruption of *Chlorella pyrenoidosa*," *Asia-Pac. J. Chem. Eng.*, **12**, 502–510 (2017).
- [17] M. Kurokawa, P. M. King, X. Wu, E. M. Joyce, T. J. Mason and K. Yamamoto, "Effect of sonication frequency on the disruption of algae," *Ultrason. Sonochem.*, **31**, 157–162 (2016).
- [18] D. Kang, Y. Jiang, L. Xing, G. Zhou and W. Zhang, "Inactivation of *Escherichia coli* O157:H7 and *Bacillus cereus* by power ultrasound during the curing processing in brining liquid and beef," *Food Res. Int.*, **102**, 717–727 (2017).
- [19] A. B. G. Loredó, S. N. Guerrero and S. M. Alzamora, "Inactivation kinetics and growth dynamics during cold

- storage of *Escherichia coli* ATCC 11229, *Listeria innocua* ATCC 33090 and *Saccharomyces cerevisiae* KE162 in peach juice using aqueous ozone,” *Innov. Food Sci. Emerg. Technol.*, **29**, 271–279 (2015).
- [20] S. Gao, G. D. Lewis, M. Ashokkumar and Y. Hemar, “Inactivation of microorganisms by low-frequency high-power ultrasound: 1. Effect of growth phase and capsule properties of the bacteria,” *Ultrason. Sonochem.*, **21**, 446–453 (2014).
- [21] S. Koda, M. Miyamoto, M. Toma, T. Matsuoka and M. Maebayashi, “Inactivation of *Escherichia coli* and *Streptococcus mutans* by ultrasound at 500 kHz,” *Ultrason. Sonochem.*, **16**, 655–659 (2009).
- [22] I. Hua and J. E. Thompson, “Inactivation of *Escherichia coli* by sonication at discrete ultrasonic frequencies,” *Water Res.*, **34**, 3888–3893 (2000).
- [23] S. Guerrero, A. Lopez-Malo and S. M. Alzamora, “Effect of ultrasound on the survival of *Saccharomyces cerevisiae*: Influence of temperature, pH and amplitude,” *Innov. Food Sci. Emerg. Technol.*, **2**, 31–39 (2001).
- [24] R. Y. Stanier, J. L. Ingraham, M. L. Wheelis and P. R. Painter, *The Microbial World* (Pearson College Div; Subsequent, London, 1986).
- [25] J. F. Wilkinson, *Introduction to Microbiology* (Blackwell Scientific Publishing, Hoboken, NJ, 1986).
- [26] M. T. Madigan, J. M. Martinko and J. Parker, *Brock Biology of Microorganisms* (Prentice Hall, Upper Saddle River, NJ, 2002).
- [27] P. Piyasena, E. Mohareb and R. C. McKellar, “Inactivation of microbes using ultrasound,” *Int. J. Food Microbiol.*, **87**, 207–216 (2003).
- [28] U. Hulsen, “Alternative heat treatment processes,” *Eur. Dairy Mag.*, **3**, 20–24 (1999).
- [29] R. F. Contamine, A. M. Wilhelm, J. Berlan and H. Delmas, “Power measurement in sonochemistry,” *Ultrason. Sonochem.*, **2**, S43–S47 (1995).
- [30] K. Yamamoto, P. M. King, X. Wu, T. J. Mason and E. M. Joyce, “Effect of ultrasonic frequency and power on the disruption of algal cells,” *Ultrason. Sonochem.*, **24**, 165–171 (2015).
- [31] T. Kimura, T. Sakamoto, J.-M. Leveque, H. Sohmiya, M. Fujita, S. Ikeda and T. Ando, “Standardization of ultrasonic power for sonochemical reaction,” *Ultrason. Sonochem.*, **3**, S157–S161 (1996).
- [32] S. Koda, T. Kimura, T. Kondo and H. Mitome, “A standard method to calibrate sonochemical efficiency of an individual reaction system,” *Ultrason. Sonochem.*, **10**, 149–156 (2003).
- [33] J. Jin, H. Kumeta, F. Takahashi and Y. Suzuki, “Characterization of sonochemical reactors by an electrochemiluminescent probe,” *Bunseki kagaku*, **59**, 51–56 (2010) (in Japanese).
- [34] T. T. Nguyen, Y. Asakura, S. Koda and K. Yasuda, “Dependence of cavitation, chemical effect, and mechanical effect thresholds on ultrasonic frequency,” *Ultrason. Sonochem.*, **39**, 301–306 (2017).
- [35] P. V. Zinin and J. S. Allen, “Deformation of biological cells in the acoustic field of an oscillating bubble,” *Phys. Rev. E*, **79**, 021910 (2009).
- [36] M. Ogino and T. Nishiumi, “Sterilization of heat-resistant spores by a combination of high-pressure and subsequent heat treatment,” *Rev. High Press. Sci. Technol.*, **25**, 334–342 (2015).
- [37] A. Kobayashi and S. Maeda, “Application of high-pressure treatment for food processing,” *J. Soc. Mater. Sci. Jpn.*, **67**, 521–526 (2018) (in Japanese).