Effects of the initial concentration of microorganisms on inactivation by ultrasonic cavitation

Kei Nishiguchi†, Shun Nagaura, Ken Yamamoto (Grad. School Eng., Kansai Univ.)

1. Introduction

In recent years, the inactivation of microorganisms by ultrasonic cavitation has been extensively researched as a promising water treatment method because it does not require secondary treatment with chemicals and has low environmental impact. In addition, inactivation by ultrasonication has the advantage that the permeation distance of ultrasonic waves in an aqueous solution is much longer than that of ultraviolet rays used in UV sterilization. However, cavitation may not be fully effective in bacterial suspensions with high concentrations because of the scattering and attenuation of ultrasonic waves and changes in the cavitation threshold associated with these suspensions. It is also known that different ultrasonic frequencies result in different intensities of sonochemical and sonophysical effects. In our previous study, ultrasonic waves with frequencies of 26 kHz to 3.6 MHz were applied to an *Escherichia coli* suspension. Thus, we reported that the highest inactivation rate of *E. coli* was observed at 430 kHz, and its inactivation mechanism was the chemical action of ultrasonic cavitation[1].

The present research was conducted to investigate the influence of the initial microbial concentration on inactivation by ultrasonic cavitation. *E. coli* suspensions with an initial cell number of 10^5 to 10^8 CFU/ml were treated with ultrasonic waves, and the inactivation rate was calculated by the colony counting method. We also quantified the sound pressures in the reactors using a hydrophone. In addition, the cell surfaces were observed by scanning electron microscopy and measured the turbidity of suspensions using spectrophotometers to characterize the ultrasonic cavitation effects contributing to the inactivation.

2. Methods

2.1 Experimental system

E. coli (NBRC108669) was selected as the target for inactivation by ultrasonic waves in this study and was incubated in lysogeny broth with shaking at 30°C for 4 h. The resulting cultures were centrifuged, and the resulting pellets were suspended in Milli-Q water (Merck Millipore, Burlington, MA). The initial concentration of microorganism suspensions was adjusted using a UV-Visible spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan) before sonication. The microorganism suspension was prepared with initial concentrations of 5.7, 6.0, 7.9, and 9.0 log CFU/ml to investigate the effect of initial concentration on inactivation by ultrasonic cavitation.

2.2 Sonication

Figure 1 shows a diagram of the experimental setup. A cylindrical stainless steel sample tank surrounded by a cooling water jacket was installed above the 430 kHz ultrasonic transducer unit driven by the oscillator (QUAVA mini; Kaijo Corp., Tokyo Japan). The bacterial suspension temperature was maintained at 20 ± 1°C by circulating cooling water through the water jacket. The bacterial suspension of 100 ml was sonicated for 30 min. The acoustic power to each suspension was measured using the calorimetric method and maintained at 10 ± 1 W.

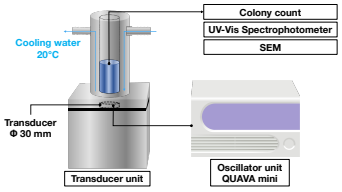

Fig. 1 Experimental apparatus used for sonication.

2.3 Analytical methods

To determine the inactivation rate of *E. coli*, the bacterial suspensions before and after sonication were incubated on agar medium and placed in an incubator at 30°C for 48 h; then, the colonies were counted. The optical density at 600 nm (OD_{600}) was measured using a UV-Visible spectrophotometer. The cell wall and cell morphology were observed by scanning electron microscopy (SEM) (TM3030Plus; Hitachi High-Tech Corp., Tokyo, Japan). The chemical effects of ultrasonic cavitation were measured by the KI method. Sound pressure measurements in the sample tank were achieved using a calibrated hydrophone (HCT-0320; Onda Corp., Sunnyvale, CA).

3. Results and discussion

3.1 Effects of the initial *E. coli* concentration

The effect of the initial *E. coli* concentration

on inactivation was investigated at 430 kHz, which was determined to be most effective for deactivation in a past study. **Figure 2** shows the inactivation rate of *E. coli* for each initial cell number of microorganisms. The inactivation rate increased with treatment time for all initial cell numbers. The inactivation rate was about 99% for 5.7 log CFU/ml and 100% for 6.0 log CFU/ml after 15 min of sonication. The highest inactivation efficiency of about 99% was obtained for 7.9 log CFU/ml after 10 min of sonication. In contrast, the inactivation rate was about 73% for 9.0 log CFU/ml bacterial suspensions sonicated for 30 min. These results suggested that the impacts of both physical and chemical effects of ultrasonic cavitation contributing to the inactivation of *E. coli* may differ among initial concentrations.

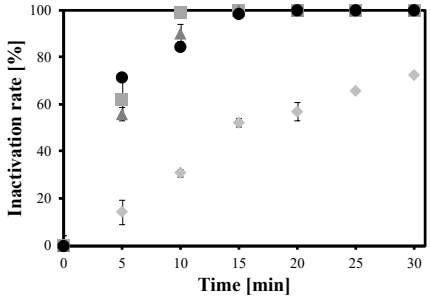

Fig. 2 Inactivation rate of *E. coli* against treatment time at an acoustic power of 10 W: ● 5.7 log CFU/ml, ▲6.0 log CFU/ml, ■7.9 log CFU/ml, and◆9.0 log CFU/ml.

3.2 Physical effects on *E. coli*

Figure 3 shows SEM images of *E. coli* before and after ultrasonic treatment at 430 kHz. No damage on bacterial surfaces was confirmed for 7.7 log CFU/ml. However, membrane ruptures and damage to the cell wall were observed for 9.0 log CFU/ml. The OD_{600} values after sonication durations for different initial cell numbers are shown in **Fig. 4**. There was no significant difference in optical density for 7.9 log CFU/ml. In contrast, the optical density for 9.0 log CFU/ml was decreased to approximately 37% by 30 min of sonication. The change in optical density was related to the concentration of cell suspension and also cell membrane permeability. Cells in 9.0 log CFU/ml suspensions may be damaged and lysed. Our hydrophone measurements of suspensions with different initial numbers of *E. coli* did not show any marked differences in the sound pressures. The increase in concentration of the heterogeneous media was generally predicted to decrease sonochemical reactions, in line with the report of Barchouchi et al. that ultrasonic power may dissipate from chemical effects to mechanical effects as a consequence of increased particle surface area in the reactor[2]. Therefore, it is suggested that cells in high-concentration cell suspensions (9.0 log CFU/ml) may be destroyed by the physical effects of ultrasonic cavitation.

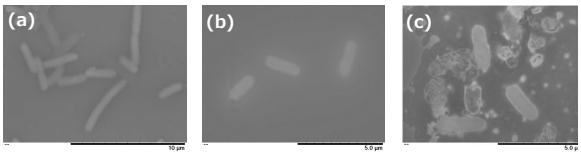

Fig. 3 Scanning electron microscopy (SEM) images of *Escherichia coli* cells before (a) and after sonication for 30 min. (b)7.9 log CFU/ml (c) 9.0 log CFU/ml.

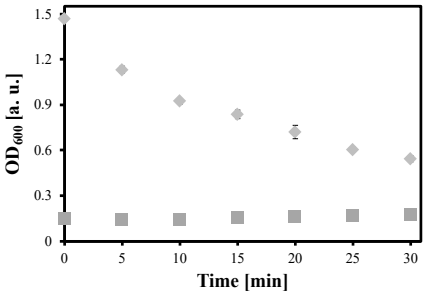

Fig. 4 Optical density (OD_{600}) of *Escherichia coli* cell suspensions against sonication time at an acoustic power of 10 W: ■7.9 log CFU/ml, and ◆9.0 log CFU/ml

4. Conclusion

Our previous studies investigated the broadband frequency dependence on inactivation of *E. coli* (initial cell number of 7.6 ± 0.3 log CFU/ml), revealing that the *E. coli* inactivation was caused by the chemical effects of ultrasonic cavitation. In the present study, we investigated the effect of initial microbial concentration (approximately 5.7, 6.0, 7.9, and 9.0 log CFU/ml) on inactivation by ultrasonic cavitation at 430 kHz, which was determined to be most effective for inactivation in previous research. These results suggested that the mechanisms of inactivation of *E. coli* by ultrasonic cavitation were mainly chemical effects at low microbial concentrations and physical effects at high microbial concentrations.

References

1. Y. Hashimoto, Y. Otani, A. Yabunaka, R. Ikeuchi and K. Yamamoto: Acoust. Sci. & Tech. **41** (2020) 877.
2. A. Barchouchi, S. Molina-Boisseau, N. Gondrexon and S. Baup: Ultrason. Sonochem. **72** (2021) 105407.