Strength and Frequency of Underwater Shock Waves Related to Sterilization Effects on a Marine Bacterium

J. Wang, A. Abe, N. Ito, and K. Nishibayashi

Abstract The paper reported on a study of sterilization effects on marine *Vibrio* sp. under different strength and frequencies of underwater shock waves generated by electric discharges. Bio-experiments were carried out with the induced bubbles behind the underwater shock waves in a cylindrical water chamber. Propagation behaviors of the shock wave in the water chamber are analyzed using an axisymmetric numerical simulation, and pressure measurement is also carried out. The generation of the bubbles is investigated using an optical method. As a result, the marine bacteria are completely inactivated in a short time, and it is clarified that sterilization effect is closely related with the number density of bubbles, pressure, and frequencies of underwater shock waves.

1 Introduction

Underwater shock wave has been extensively applied in the fields of medical engineering, food sciences, material processing, and other industries. Abe et al. [1] proposed the application of underwater shock wave to the sterilization of ships' ballast water by using microbubble motion. To investigate the potential of this shock sterilization method, Wang and Abe [2] carried out a bio-experiment of marine *Vibrio* sp. using the underwater shock waves with a frequency of 1 Hz and the microbubbles produced by a bubble generator in a circular-flow water tank. They clarified a high sterilization effect in the presence of both the shock waves and microbubbles and also pointed out that cavitation bubbles generated behind the focus of the underwater shock wave showed a potential for the inactivation of

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marine bacteria. Hence, they produced a cylindrical water chamber to understand the contribution of the cavitation bubbles to the sterilization [3]. Cavitation bubbles could be produced by the concentration of the reflected underwater shock waves at the wall of the cylindrical water chamber, and an effective sterilization was obtained in a short time. The sterilization mechanism of these cavitation bubbles is as follows: bubbles produced by the concentration of the underwater shock waves begin collapsing motion just after they interact with the reflected shock waves at the inner wall of the water chamber or the next coming shock waves. Eventually, rebound shock waves and free radicals are generated by the collapse of cavitation bubbles, and marine bacteria near the bubbles are killed biochemically and mechanically.

In the paper, to clarify sterilization effect of underwater shock waves with the induced bubbles, bio-experiments with marine *Vibrio* sp. are carried out under different conditions of electric discharges in a cylindrical water chamber. Propagation behaviors of the underwater shock wave are numerically analyzed using an axisymmetric TVD scheme. On the other hand, pressure measurement is also carried out at the central axis of the water chamber. In addition, an optical observation with a video camera is used to investigate the generation of bubbles in the water chamber. The sterilization effects are discussed using different strength and frequencies of the underwater shock waves.

2 Bio-Experimental Setup

Figure 1 shows a bio-experimental arrangement. Experimental devices consisted of a cylindrical water chamber [4], a high-voltage power supply generating the shock waves (HPS 18 K-A, Tamaoki Electronics Co., Ltd.), a pulse generator, and a system of pressure measurement including a fiber optical probe hydrophone (FOPH 2000) and an oscilloscope. This cylindrical water chamber consisted of two equivalent parts, namely, the upper part was a test chamber and the lower part was an electric discharge chamber. A silicone film of 0.1 mm thick was used to separate the two chambers. Cell experiments with marine *Vibrio* sp. were carried out in the test chamber. In the experiments, samples extracted from the water chamber was diluted

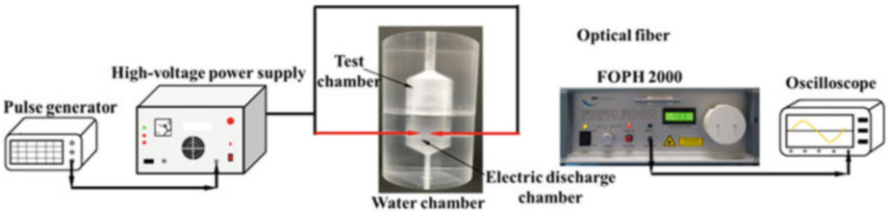

Fig. 1 Schematic of experimental setup with cylindrical water chamber

serially and then spread on agar plates. The plates were incubated for 24 h at 30 °C. Finally, the cell viability in 1 ml was evaluated by the numbers of colony-forming cells in agar plates on the basis of the dilution ratio.

3 Results and Discussion

Based on the study by Wang and Abe [3], an axisymmetric numerical simulation was used to analyze the propagation of an underwater shock wave in the cylindrical water chamber. This simulation code was executed by a second-order accurate implicit TVD finite differential scheme, and Tait equation was used as the equation of the state for water in this code. The underwater shock wave was generated by the instant release of high pressure in a small dense water sphere shown in Fig. 2 (1). The initial density ratio to the ambient, the initial pressure ratio, and the diameter of the water sphere were decided to 1.030, 728, and 3.5 mm in this simulation by comparison with the experimental pressure attenuation of a spherical expanding underwater shock wave in their study [3]. In Fig. 2 (2), the spherical underwater shock wave was propagating in the water chamber, reflected at the inner wall, and concentrated at the central axis. Next, it was found the concentrated shock waves propagated following negative pressure region, so that the tensile stress regions were clearly found behind that. Cavitation bubbles were thought to be generated in the tensile stress region.

Figure 3 shows time variations of the pressure obtained from experimental measurement and numerical analysis at 30 mm from the discharge point. The black line indicates the results of experimental data and the green line for the numerical analysis. In the figure, the first and second waves were found at the same timing between the experimental and numerical results, but they did not agree quantitatively. Furthermore, after 35 μs there was no coincidence between them. In particular, all the negative pressures were estimated to be larger in the numerical result. These quantitative differences were caused by the use of Tait equation, and cavitation generation was not assumed in this simulation. In the experimental profile, pressure of the second shock wave was lower than that of the first wave. The result was probably caused with the little movement of the discharge position

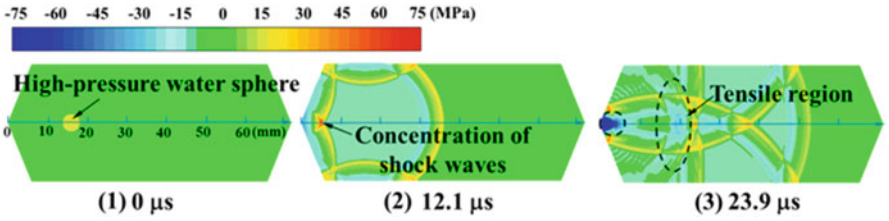

Fig. 2 Numerical pressure distribution in water chamber

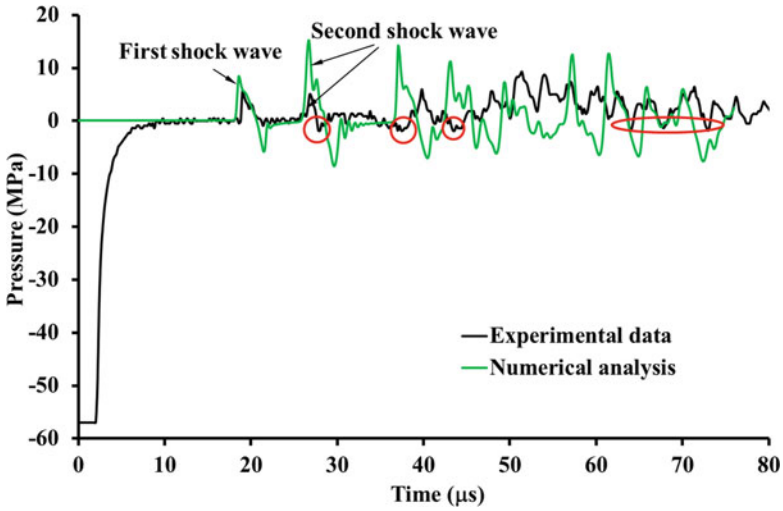

Fig. 3 Comparison of pressure records between experimental measurement and numerical analysis at distance of 30 mm from discharge point

and the inner-wall condition of the water chamber, and so on. The tensile regions marked by the circles were clearly observed in the experimental result. These strong tensile stresses could cause the generation of cavitation bubbles, so that the pressure variations were affected by the motions of the bubbles after 30 μs in the experimental profile. However, the effect of the cavitation bubble was not considered in the numerical simulation; therefore the pressure variations were different after the second shock wave.

Figure 4 shows estimation of the number of viable cells after an electric discharge of 31.6 kV in the test chamber. The initial density of marine *Vibrio* sp. was about 4.62×10^4 cfu/ml. The solid squares on the red curve represent the reference data obtained from the solution of the marine bacteria without the action of any shock waves. The number of viable cells hardly changed throughout the course of the experiment. The solid diamonds on the blue curve present the results of the bio-experiment predicted by the colony number of viable cells grown on the agar plates, as shown in the photos. Here, the electric discharge was triggered once every second, i.e., the applied frequency of the shock waves was 1 Hz. Samples were taken from the test chamber at 30, 60, 90, 100, and 110 s, diluted serially, and then spread on the agar plates. The agar plates in the photos had the same dilution. It was found that the gradient of the curve increased gradually after every sample extraction. Finally, the marine bacteria were found to have been killed completely after about 100 s. This result indicates that the sterilization efficiency increased gradually after every sample extraction. The pressure of the shock wave at each electric discharge is thought to be constant. Hence, this suggests that the number density of the bubbles can increase with the sample extraction.

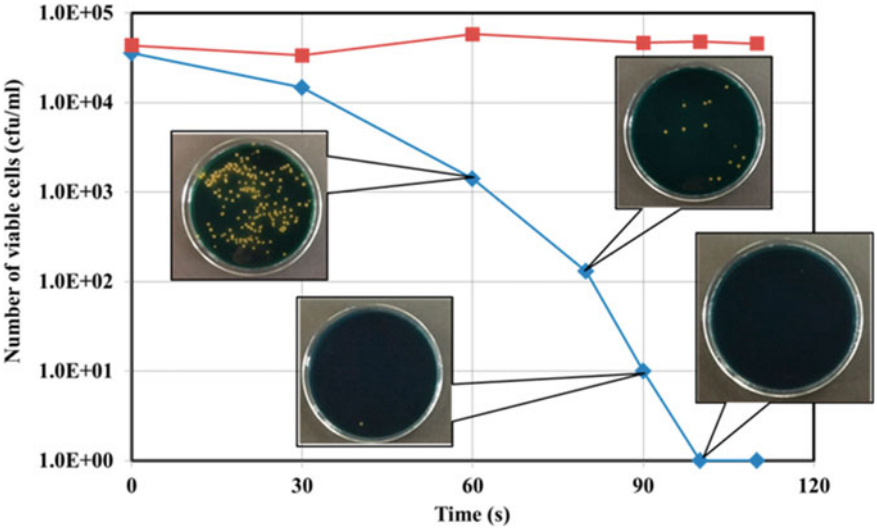

Fig. 4 Estimation of number of viable cells at electric discharge of 31.6 kV: ■ without action of shock waves, ◆ with the action of shock waves

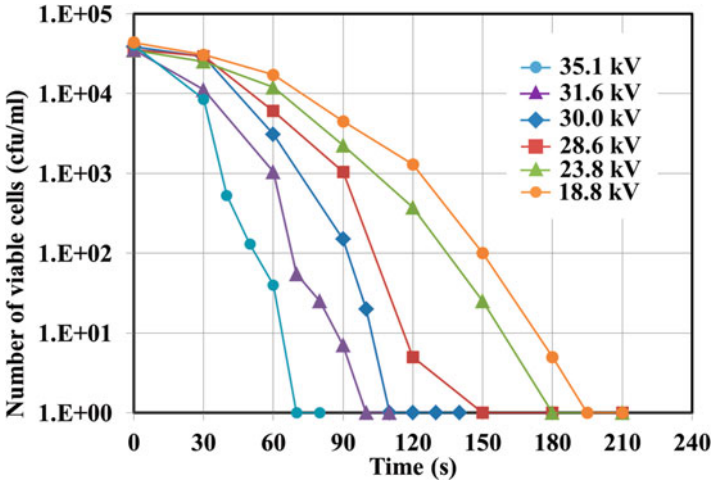

Fig. 5 Bio-experimental results obtained with electric discharge of 35.1 kV–18.8 kV

Figure 5 shows estimates of the number of viable cells obtained with an electric discharge of 35.1 kV–18.8 kV. The applied frequency of shock waves was 1 Hz. The plots shown in this figure are of the averages for six sets of bio-experimental data. The initial concentration of the marine bacteria was about 4.85×10^4 cfu/ml. From this figure, the marine bacteria were completely killed after about 70 s with an electric discharge of 35.1 kV. In the case of an 18.8-kV discharge, the time

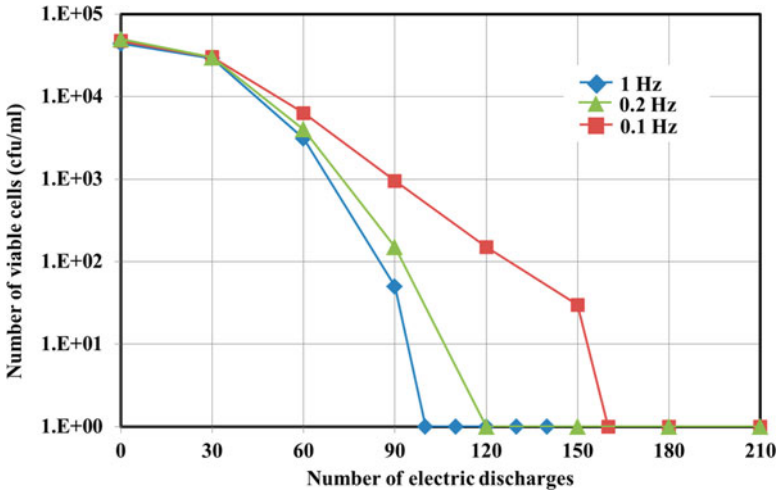

Fig. 6 Estimation of number of viable cells at electric discharge of 31.6 kV. ◆ 1 Hz, ▲ 0.2 Hz, and ■ 0.1 Hz

required to attain a complete sterilization was about 190 s. Thus, we can say that the sterilization effect increased with the output power of the electric discharge. The resulting shock pressures are mainly responsible for these results because the pressures behind the shock wave fronts increase with the output power. On the other hand, from the results obtained with an electric discharge of 30.0 kV to 18.8 kV, the gradients of the curves were found to be almost the same during the first 30 s, after which the differences in the sterilization effect arose. This suggests that the effective sterilization was not induced during the first 30 s. The reason for this could be that a significant number of bubbles had not been generated in the test chamber before the first extraction.

Figure 6 shows estimates of the number of viable cells at an electric discharge of 31.6 kV under different applied frequencies of the shock waves. The solid diamonds, triangles, and squares represent the results of the bio-experimental obtained with 1 Hz, 0.2 Hz, and 0.1 Hz, respectively. The other experimental conditions were the same as that in Fig. 5. This figure shows that the number of the electric discharges at which perfect sterilization is obtained increased as the applied frequency of the shock waves decreased. As shown in Fig. 6, the gradients of the three curves were almost the same during the first 30 shoots. This suggests that the strength of the collapsing motion were the same during this period, i.e., the generated bubbles in the test chamber could almost disappear before the arrival of the next shock wave. Given the results shown in Figs. 2 and 3, these bubbles were thought to be generated by the concentration of the reflected shock waves. Subsequently, the differences in the sterilization effect were apparent. These differences indicate that many bubbles remained upon the arrival of the next shock wave with frequencies of 1 Hz and 0.2 Hz. These remaining bubbles were thought to be other than

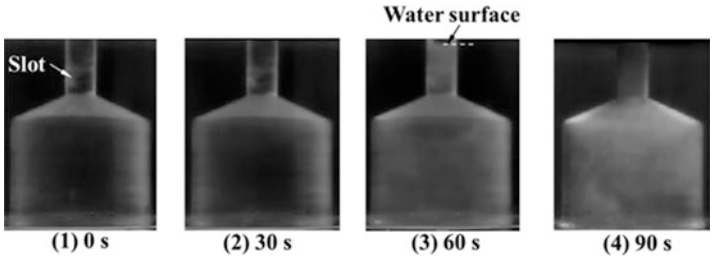

Fig. 7 Photos of test chamber at 31.6-kV electric discharge with sample extraction

the cavitation bubbles generated behind the reflected shock waves, given that the lifetimes of cavitation bubbles are in the order of millisecond. From those experimental situations, it was assumed that these bubbles were produced by other causes.

Figure 7 shows photos of the test chamber with an electric discharge of 31.6 kV before the sample extractions. Due to the light refraction and low transparency of the cylindrical water chamber, the states in the chamber were a little unclear. However, we could recognize the generation of the bubbles by the change of the white area in the photos. By comparing with the photo at 0 s, we can see that the state in the test chamber has barely changed from that in Fig. 7 (2). At 60 s, the number of remaining bubbles increased, and then, at 90 s, the test chamber became full of generated bubbles. During the experiments, the water surface in the slot went slightly down with every sample extraction of 0.1 ml taken from the cell suspension. However, the volume of the extracted samples was small in contrast to the total volume of the cell suspension in the test chamber, so that it can be negligible. From the observation of the test chamber, the rapid rise-and-fall motion of the water surface in the slot was found with the triggers of the electric discharges. At this time, air in the slot was brought into the cell suspension, and simultaneously tremendous bubbles were observed in the test chamber. The remaining bubbles at 60 s and 90 s in Fig. 7 were thought to be generated in this way. The movement of the water surface could be caused by the oscillation of the silicone film between the two chambers. The underwater electric discharge produces a vapor bubble between the electrodes after the generation of the shock waves. The vapor bubble contracts following expansions so that the collapsing motion of the vapor bubble resulted in the oscillation of the silicone film and then the generation of the bubbles in the test chamber.

4 Conclusions

The sterilization effects on marine *Vibrio* sp. were investigated with the underwater shock waves and induced bubbles in a cylindrical water chamber. Bio-experiments were carried out under different strength and frequencies of the shock waves

generated by the electric discharges. The numerical analysis showed that the shock wave propagating in the water chamber was reflected at the inner wall of the water chamber and then concentrated at the central axis of the cylinder and also predicted the generation possibility of cavitation bubble generation behind the reflected shock waves. Moreover, the shock wave-cavitation interaction was also confirmed in experimental pressure profile. Given the optical observation, the generated bubbles in the test chamber contained the cavitation bubbles behind the concentration of the reflected shock waves and bubbles produced by the oscillation of the silicone film between the two chambers due to rapid expansion and contraction of a vapor bubble at the electrodes. It was found that the bubbles resulting in the effective sterilization were from the latter. The results of the bio-experiments showed the marine bacteria were completely killed in a short time, and a large number density of bubbles, a high pressure, and a high frequency of incident shock wave give rise to an excellent sterilization effect. There is a possibility of a high sterilization effect in the presence of underwater shock wave alone.

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