Study of mechanical and chemical effects induced by shock waves on the inactivation of a marine bacterium

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Summary. The present paper reports a study on the shock sterilization technique of ships' ballast water. In order to expose cell suspension to strong shock waves, a gas gun experiment was carried out. In this experiment, shock waves in the suspension of a marine *Vibrio* sp. were generated by collision between an aluminum suspension container and an impactor plate accelerated by the gas gun. The changes of shock pressure in suspension were measured by piezofilm gauges and compared them to computational results. The visualization of shock waves in water was carried out by shadowgraph method, and the marine *Vibrio* sp. was observed with an electron microscope. In addition, the affect of free radicals was also investigated using VitC-Na 2 %. It was found that both the dynamic action of shock pressure and the chemical reaction in suspension induced by shock waves were closely related to inactivation of the marine *Vibrio* sp. at an impact velocity lower than about 200 m/s.

1 Introduction

Technological development of treatment of ships' ballast water is an important assignment to preserve the marine environment [6]. The International Marine Organization (IMO) adopted the guidelines of the "International Convention for the Control and Management of Ship's Ballast Water and Sediments" in 2004, and has been implementing a global ballast water management program. Recently, many studies on the management and treatment technique of ships' ballast water have been reported all over the world (for example, see [8]). However, most of studies were intended for marine plankton. The authors consider that it will be important for the treatment of ships' ballast water to sterilize cholera bacteria in the near future. They have started the experimental study on behavior of a marine *Vibrio* sp. exposed to shock waves. In order to exert strong shock waves on the cells, a suspension container made of aluminum was collided with an aluminum impactor plate accelerated by a gas gun. In the previous study, the authors have obtained that the marine *Vibrio* sp. in a suspension container was completely inactivated at an impact velocity larger than about 110 m/s, and then the peak pressure value in the suspension container was more than 200 MPa [1]. However, inactivation mechanism of the marine cells has not been clarified enough.

In the present paper, the propagation process of shock waves in a suspension container was investigated by a numerical simulation and experimental pressure measurements. In order to discuss the mechanical and chemical effects of shock waves on the inactivation of a marine *Vibrio* sp., the damage of a cell wall after shock wave loading was confirmed by a biochemical method and an electron microscopic observation, and the generation of free radicals in suspension was also examined experimentally using VitC-Na 2%. From the above-mensioned results, the contribution of dynamic action of shock wave pressure and chemical reaction in suspension to inactivation of the marine *Vibrio* sp. was discussed.

2 Experimental

2.1 Gas gun and projectile

In this experiment, cell suspension was closed in an aluminum container and compressed by strong shock pressure. The shock pressure was generated by the impact between the suspension container and an aluminum impact plate. The impact plate is an aluminum disk 1 mm in thickness and 38 mm in diameter, and it is attached to the front surface of a projectile accelerated up to an arbitrary velocity by a single-stage gas gun. The gas gun consists in a high pressure chamber, a launch tube, and an impact chamber. The bore of the launch tube is 40 mm and its length is 2 m. The maximum pressure of driver gas in the high pressure chamber was 2 MPa. The impact velocity of projectile was measured by a magnet flyer method. A projectile was made of ABS resin.

2.2 Suspension container

A suspension container was made of aluminum, and its shape was columnar 30 mm in diameter. In the body of container, there was a cavity space to maintain 0.4 ml suspension. The wall thickness of the impact part of the suspension container was 5 mm. In order to improve the precision of plane impact between an impact plate and the suspension container, the container was fixed in a target holder, and set at the exit of the launch tube, as shown in Figure 1. The shock pressure in the suspension was measured by a piezo-film gauge (PVF_2, 11-.125-EK, Dynasen Inc.). The pressure-sensitive area of the gauge is about 9 mm^2, and its thickness is 28 μm.

In the visualization experiment, the rectangular water container with 14 mm $\times$ 20 mm acrylic windows was used. Optical observation of shock waves in the water container was carried out by shadowgraph method.

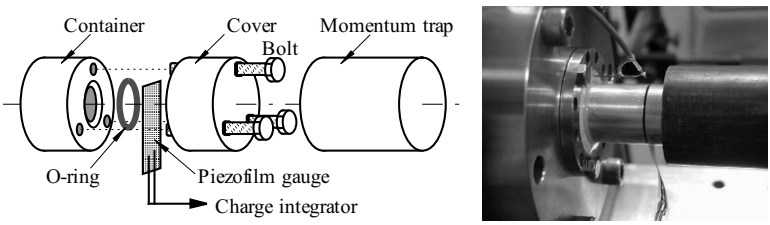


Fig. 1. Assembly and arrangement of a target set

2.3 Judgment of inactivation of marine bacteria

The marine *Vibrio* sp. used in this research belongs to the same generic group of cholera bacteria that was regulated by the international convention for ballast water management. We isolated the marine *Vibrio* sp. from seawater, and cultivated colonies using artificial seawater. Inactivation of the cells after an impact was decided by counting colonies on an agar plate. In addition, the agar plate containing sodium cholate was also used to investigate damage of the outer membrane of cells.

3 Numerical simulation

In order to predict the propagation process of shock waves in cell suspension, the shock wave phenomena generated in a suspension container were calculated by a general purpose transient dynamic finite element program (LS-DYNA, LSTC). The elastic and plastic deformation of aluminum container was represented using the Johnson-Cook model [7]. The Johnson-Cook equation is given by

$$\sigma = (C_1 + C_2\epsilon^N)(1 + C_3 \ln \epsilon^*)(1 - T^{*M}), \tag{1}$$

where σ is the equivalent yield strength, ϵ is the equivalent plastic strain, ϵ^* is the dimensionless plastic strain rate for the reference strain rate (usually equal to 1.0 s^{-1}), and C_i, N and M are constants of the material. T^* is the dimensionless temperature, and it is given by

$$T^* = \frac{T - T_{room}}{T_{melt} - T_{room}}, \tag{2}$$

where T is the current temperature, T_{room} is the ambient temperature, and T_{melt} is the melt temperature. The ABS resin used for the projectile was assumed to be a perfectly elastic material.

For the equation of state of the cell suspension, the Tait equation was used. The Tait equation [3] is defined as

$$P = \alpha \left[\left(\frac{\rho}{\rho_0} \right)^{\beta} - 1 \right], \tag{3}$$

where P is the gauge pressure, ρ is the density, ρ_0 is the initial density, and α and β are constants. In this equation, $\alpha = 304.7$ MPa and $\beta = 7.15$ were used for 0.7 mol/kg of salt water. In order to use the equation 3 in the calculation code, it was transformed to the following polynomial approximation,

$$P = 12951\eta^3 + 6671.2\eta^2 + 2178\eta, \tag{4}$$

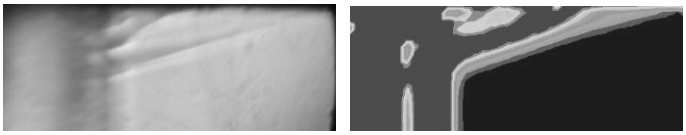
where η, $= \rho/\rho_0$ - 1, is compression strain.

4 Results and discussions

4.1 Shock wave generation in a suspension container

Figure 2 shows a comparison between an experimental shadowgraph and numerical pressure distributions obtained at 400 m/s impact velocity. Figure 2(a) shows that a plane underwater shock wave has occurred at the left side boundary, and oblique shock waves generated by the precursive elastic and plastic shock waves in the aluminum frame have propagated from the upper side boundary. From these results, we could obtain good agreements on the position of the plane underwater shock wave and the inclined angle of the first oblique shock wave.

Figure 3 shows a comparison between experimental and numerical pressure changes at 96.2 m/s impact velocity [2]. These results indicate good agreements until about 15 μs. However, the experimental pressure decreases gradually after 15 μs, while the numerical result keeps around 150 MPa. The difference shows the leakage of pressure from the suspension container in this experiment. From the above-mentioned results, it was confirmed that the pressure change generated in suspension by the first shock wave would be simulated quantitatively by the numerical calculation.



(a) (b)

Fig. 2. Experimental shadowgraph (a), and computational pressure distributions (b), obtained at 400 m/s impact velocity

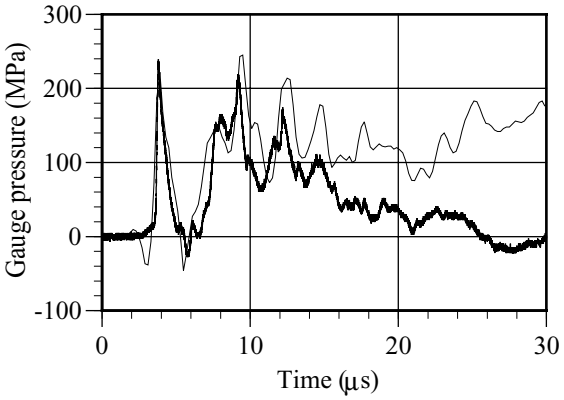
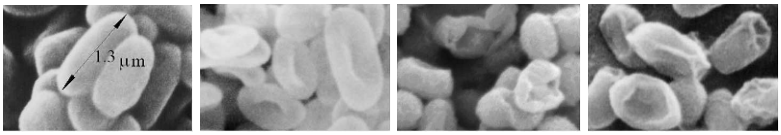


Fig. 3. Comparison of pressure changes: a thin line is computational result, and a bold line is experimental one

4.2 Electron microscopic observation

The marine *Vibrio* sp. was observed with an electron microscope. Figure 4(a) shows an image of the cells before the shock event. It is found that the configuration of a marine *Vibrio* sp. is originally a spheroid of about 1 μm in the major axis. Figure 4(b) and 4(c) are the samples of inactive cells obtained at a low impact velocity less than 100 m/s and at a 266 m/s impact velocity, respectively. It is found that the configurations of inactivate cells after the low velocity impact became like that of red blood cells, while the burst cells were observed at the high impact velocity. In addition, the osmotic pressure of 2 mol NaCl for four hours shriveled the outer membrane of the cells.



(a) (b) (c) (d)

Fig. 4. Images of a marine *Vibrio* sp. obtained by an electron microscope: picture (a) is the control, and others are the samples of inactive cells obtained at a low impact velocity less than 100 m/s (b) and at a 266 m/s impact velocity (c), and osmotic pressure (d)

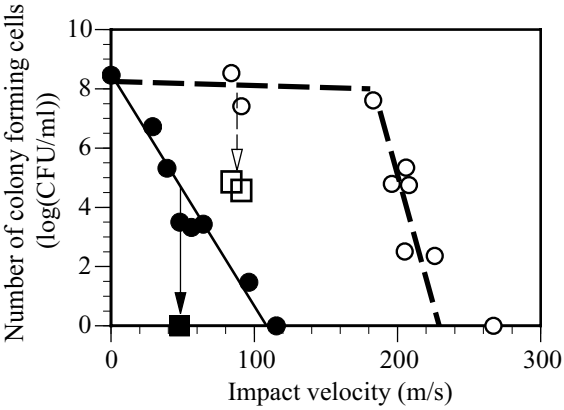


Fig. 5. Relation between survival number of cells and the impact velocity: solid circles indicate the results using normal suspension, a solid square is the result using culture media containing sodium cholate, open circles are the results of addition VitC-Na 2 % to normal suspension, and open squares are the results using the antioxidant agent and culture media containing sodium cholate

4.3 Inactivation effects of shock pressures

The relation between the survival number of the marine *Vibrio* sp. and the impact velocity is shown in figure 5 using solid circles and a solid line [2]. The number of cells decreases with an increase of the impact speed, and the inactivation of the cells are achieved completely at an impact speed larger than about 110 m/s. The peak gauge pressure obtained at 110 m/s in the present suspension container measured about 200 MPa. A solid square in this figure shows the result using an agar plate containing sodium cholate that completely inactivates the marine *Vibrio* sp. damaged on the outer membrane [4,5]. The 10^3 CFU/ml of the cells obtained by the impact velocity of about 49 m/s resulted in the complete inactivation using the sodium cholate. Therefore, it was appeared that the shock pressures generated at an impact velocity lower than 100 m/s gave the damage to the outer membrane of the marine cells.

As shown in figure 3, strong negative pressure has been measured immediately after the arrival of the first shock wave. It was seemed that movement of the cover of suspension container by an inner pressure loading caused the negative pressure, and it was suspected that cavitation bubbles were generated in the container. If cavitation bubbles collapse in the container, the free radicals are produced by the collapse energy of them and act on the marine bacteria. In order to investigate the effects of radicals, the experiment was carried out using the suspension including VitC-Na 2 % that is a representative antioxidant agent. The open circles and the broken lines in figure 5 show the experimental results. It was found that the inactivation of the marine cells was apparently inhibited by the VitC-Na to an impact velocity lower than about 200 m/s. In this case, the complete inactivation effect was obtained at an impact velocity larger than about 250 m/s.

The open squares in figure 5 show the results obtained at the impact velocity about 88 m/s using the suspension added an antioxidant agent and an agar plate containing sodium cholate. In this case, the survival number of cells decreased from 10^8 to 10^5 CFU/ml. The result suggested that the survival cells that had been protected from the

radicals using an antioxidant agent damaged dynamically on their cell wall by shock pressures.

5 Conclusions

The dynamic and chemical effects of shock pressures to the inactivation of a marine *Vibrio* sp. were examined by the shock experiment using a gas gun. From the electron microscopic observation of the shocked cells, it was shown that the configurations of inactive cells were significantly different at the impact velocities. The inactive cells obtained by a low velocity impact showed the configuration like the red blood cells, and the burst of the cells was observed at the high impact velocity. On the other hand, we could not obtain the complete inactivation of the marine *Vibrio* sp. using the suspension with an antioxidant agent at an impact velocity lower than 200 m/s. However, the damage of the outer membrane of survival cells was obviously shown by the bio-experiments using the sodium cholate. Therefore, we consider that the dynamic effects of shock waves will be dominantly at the high impact velocity, but at the low impact velocity dynamic and chemical effects by shock pressures and free radicals contribute to the inactivation of the cells.

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