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(Citation)

Shock Waves, 17(1-2):143-151

(Issue Date)

2007-08

(Resource Type)

journal article

(Version)

Accepted Manuscript

(URL)

<https://hdl.handle.net/20.500.14094/90000589>



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Received: date / Accepted: date

Abstract The effect of shock pressures on the inactivation of a marine *Vibrio* sp. was studied experimentally and numerically. In the experiment, an aluminum impactor plate accelerated by a gas gun was used to induce shock waves in a sealed aluminum container with cell suspension liquid inside. The shock pressures in the container were measured by a piezofilm gauge. Several tens to hundreds megapascals (MPa) of pressure were measured at the shock wave front. An FEM simulation, using the Johnson-Cook model for solid aluminum and the Tait equation for the suspension liquid, was carried out in order to know the generation mechanism of shock pressures in the aluminum container. The reflection, diffraction and interaction of shock waves at the solid-liquid boundaries in the aluminum container were reasonably predicted by the numerical simulation. The changes in shock pressures obtained from the computational simulation were in good agreement with those from the experiment. The number of viable cells decreased with the increase of peak pressures of the shock waves. Peak pressures higher than 200 MPa completely inactivated the cells. At this pressure, the cell structures were deformed like the shape of red blood cells, and some proteins leaked from the cells. These results indicate that the positive and negative pressure fluctuations generated by shock waves contribute to the inactivation of the marine *Vibrio* sp.

Keywords Shock Pressures · Gas Gun · Inactivation · Marine *Vibrio* sp.

PACS 47 · 40 · Nm

1 Introduction

Ship ballast water has been causing environmental problems for the marine ecosystem [1, 2]. In general, ballast water is used to control the draft and provide stability. The ballast water is pumped up into the ship's ballast tanks when cargos are unloaded from a ship, while the ballast water is pumped out when cargos are loaded on a ship. Meshes are usually used at the ship's ballast water intake to screen out many kinds of marine life. However, marine microorganisms smaller than several centimeters often get into ballast water tanks through the meshes, and then these organisms are transferred to other places. The amount of ballast water transferred globally is estimated to be about three to ten billions tons in a year. As a result, many kinds of the microorganisms carried with ballast water are spread to non-native ocean areas, and there they might cause harm to the native living aquatic resources and bring about economic damage. 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 is implementing a global ballast water management program. Now, many studies on the management and treatment technique of ship ballast water have been conducted all over the world [3–5]. However, no current technologies can achieve a practical and satisfactory level for ballast water treatment.

We have researched the effect of shock pressures on a marine *Vibrio* sp. [6–8]. In the fields of impact engineering and food science, there have been several experimental studies on the inactivation of microorganisms by shock waves. Teshima et al. [9, 10] exposed *Escherichia coli* and lambda phage DNA in suspension liquid to pressure pulses up to 14 MPa by a diaphragmless shock tube.

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They showed the relation between the cell destruction ratio and peak pressure, and it was found that the ratio increased remarkably at a peak pressure above 12 MPa. Loske et al. [11, 12] exposed the bacteria to hundreds of underwater shock waves using an electrohydraulic shock wave generator. The survival population of *Escherichia coli* was reduced exponentially by 2,000 of applied shock waves, of which the average peak positive pressure was about 45 MPa. However, the inactivation mechanism of bacteria by shock waves has not been clear. In addition, there have been few studies on the effect of shock and dynamic pressures on bacteria in an electrolyte including a sodium ion.

The purpose of this study is to examine the effect of shock pressures on the inactivation of a marine *Vibrio* sp. by using strong shock pressures generated by a single shot with a gas gun in a suspension liquid. The inactivation of the marine bacteria was evaluated by plate counting of the viable cells. The behavior of shock waves in an aluminum container was predicted by a finite element method (FEM) numerical simulation, and the computational pressure fluctuations at a gauge position were compared with the experimental results. The morphological damage of cells and the leakage of protein from the cells were also evaluated.

2 Experiments

2.1 Experimental method

A gas gun is used to generate strong shock pressures in the suspension liquid inside an aluminum container [7, 13]. An aluminum impactor plate is accelerated by a gas gun, and it collides with the target container. Figure 1 shows an illustration of the experimental apparatus.

The launch tube diameter of the gas gun was 40 mm, and the length was 2 m. The maximum pressure in the high-pressure reservoir was 2 MPa. A projectile was made of ABS resin, and an aluminum impactor plate of 38-mm diameter and 1-mm thickness was mounted on the projectile nose, as shown in Fig. 2. A ferrite magnet of 10-mm diameter and 3-mm thickness was embedded in the projectile for measurement of the impact velocity. The impact velocity was measured by the electromagnetic method, in which the arrival times of the projectile were detected by two magnet coils near the muzzle. The magnet coils were located 70 mm and 130 mm ahead of the impact surface of the target container, respectively.

Figures 3(a)-(c) show schematic diagrams of the target container, the assemblies of the target part, and the target holder, respectively. The aluminum container was 30 mm in diameter and 20 mm in thickness, and the volume of the suspension liquid was 0.4 ml. The container was closed tightly with three bolts and an o-ring. A piezofilm gauge (PVF₂ 11-125-EK, Dynasen Inc.) was

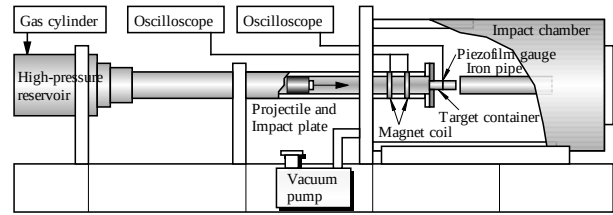
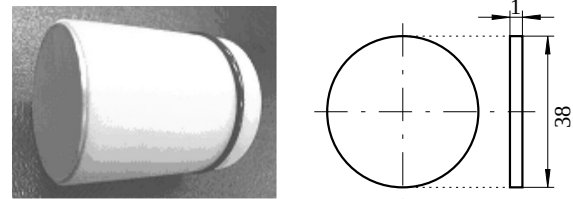


Fig. 1 Experimental apparatus



(a) Projectile with the im- (b) Aluminum impactor
pactor plate plate. Units: mm

Fig. 2 Photograph of the projectile and a schematic diagram of the impactor plate

set on the surface of the cover to measure the shock pressures in the suspension liquid. In a preliminary experiment, it was confirmed that the containers could remain leakproof after the impact experiments, even at an impact velocity faster than 300 m/s. The target aluminum container was set in the target holder, as shown in Fig. 3(c). A trigger pin sensor was set at the impact surface of the target holder to start the pressure gauge. An aluminum column serving as a momentum trap was set behind the container to reduce the impact damage to the container. The target holder assembly was placed at the exit of the launch tube, and an iron pipe was set behind the target holder to catch the target container without causing any damage to the container.

2.2 Preparation of the marine *Vibrio* sp. and estimation of the activation

The marine *Vibrio* sp. used in this research belongs to the same generic group of cholera bacteria that is regulated by the international convention for ballast water management. We isolated the marine *Vibrio* sp. from seawater, and cultivated colonies for the experiment. The artificial seawater used in this experiment consisted of dissolved NaCl (0.4 M), KCl (10 mM), CaCl₂·2H₂O (10 mM), MgCl₂ (53 mM), and Na₂SO₄ (28 mM). The culture solution for the marine *Vibrio* sp. was prepared by adding Bacto peptone (5 g/l) and yeast extract (1 g/l) into the artificial seawater. 2 ml of the suspension liquid of cultivated cells was diluted with 18 ml of the culture solution. We used this suspension liquid as the specimen in the experiment.

Inactivation of the cells after impact was decided by the plate counting method [14]. Briefly, the cell suspen-

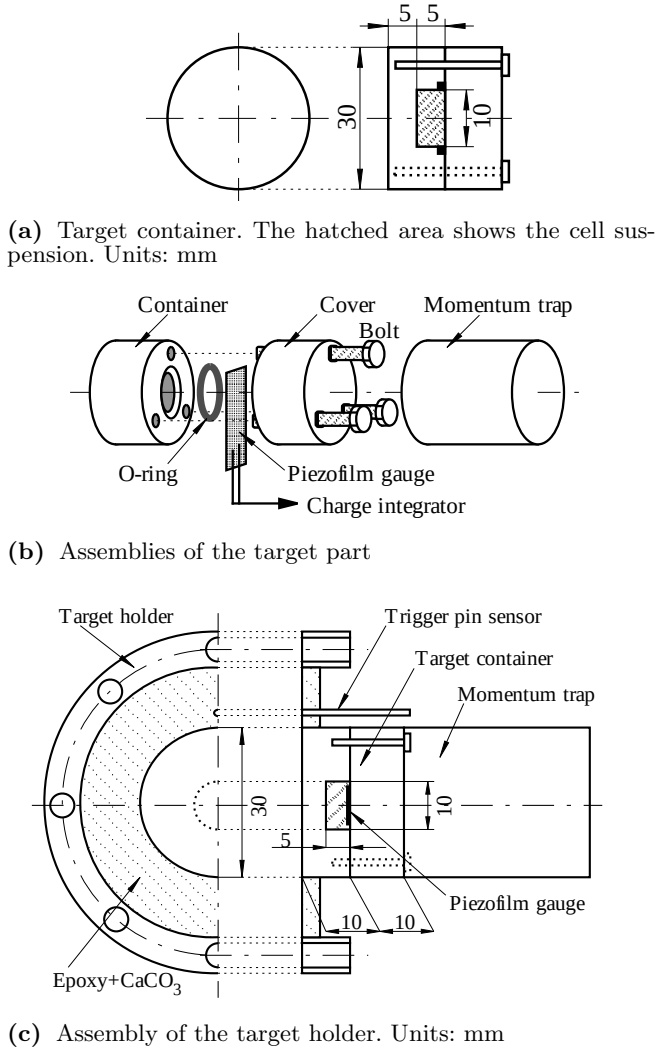


Fig. 3 Schematic diagrams of the target container, the assemblies of the target part, and the target holder

sion exposed to shock pressures was diluted serially, and then spread onto agar plates. The plates were incubated at 30 degrees Celsius for 24 hours. Colonies made on a plate (see Fig. 4) were counted. The total number of colony forming cells in 1 ml of the cell suspension after the shock event were evaluated based on the dilution ratio of the cell suspension and the number of colonies on the plate. The results were compared with those obtained from the control to make clear the effect of shock pressures on the inactivation of the cells.

Damage of the cell wall was determined by microscopic observation of the cells using an electron microscope (XL-30CP, FEI company), and measurement of the amount of protein leakage from the cells was determined using the BCA protein assay reagent (PIERCE).

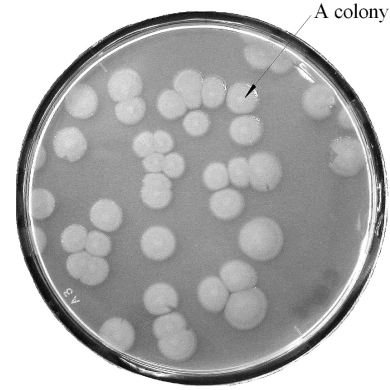


Fig. 4 Colonies made on an agar plate. A colony represents one colony forming units (CFU)

3 Numerical simulation

3.1 Constitutive relation and equation of state

A three-dimensional numerical simulation that calculates the dynamic behavior of pressure waves generated in both solids and liquids is required in order to consider the reflection, diffraction and interaction phenomena of pressure waves in the target container. These phenomena were calculated by a general purpose transient dynamic finite element program (LS-DYNA, LSTC). A Johnson-Cook constitutive relation was used in this calculation in addition to the Mie Grüneisen equation of state to predict the wave phenomena, including the elastic-plastic deformation, in the aluminum container. The Johnson-Cook equation [15] is written as

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

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

$$T^* = \frac{T - T_{room}}{T_{melt} - T_{room}}, \quad (2)$$

where T is the current temperature, T_{room} is the ambient temperature, and T_{melt} is the melt temperature. The specific values of the constants are listed in Table 1 [16,17].

The ABS resin used for the projectile is assumed to be perfectly elastic material. Properties of the ABS resin are shown in Table 2.

For the equation of state of the suspension liquid, the Tait equation was used. The Tait equation [18] is defined as

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

Table 1 Material values of aluminum, and the coefficients and constants for the Johnson-Cook model

Density (kg/m ³)	2785
Young's modulus (MPa)	72000
Elastic shear modulus (MPa)	26500
Poisson's ratio	0.32
Grüneisen parameter	2.00
C_1 (MPa)	452.4
C_2 (MPa)	457.1
C_3	1.085×10^{-2}
N	0.3572
M	1.131
T_{room}	299.15
T_{melt}	660.37

Table 2 Material properties of ABS resin

Density (kg/m ³)	1170
Young's modulus (MPa)	2300
Poisson's ratio	0.38

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.

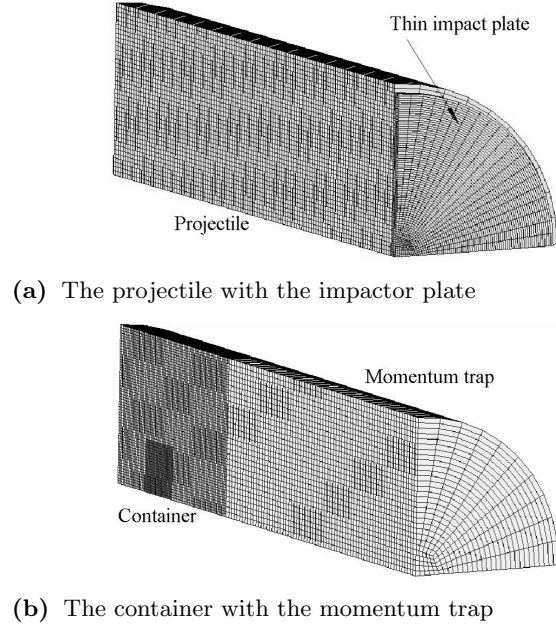
3.2 Computational condition and grid

The conditions set for the FEM grid model of the projectile, the impact plate, the container, and the momentum trap for the numerical simulation were the same as those of the impact experiment. Figure 5 shows the grid models. We should note that the grid model of the target container has no interface between the body and cover of the container. The calculation area was taken as a quarter of the total area because the configurations of all the grid models had rotational symmetry. The total number of elements used in the calculation was 171,080. The impactor plate was initially set on the nose of the projectile, and the momentum trap was initially set behind the container. The computation was started by the input of an arbitrary impact velocity to both the projectile and the impactor plate.

4 Results and discussions

4.1 Generation of shock pressures in target container

Figures 6(a)-(e) show the hydrodynamic pressure distributions in both the aluminum and the liquid parts of the container obtained by the numerical simulation. The impact velocity V_p is 96.2 m/s. Each picture shows the upper half of the cross-sectional surface of the container from the axis of rotational symmetry. As seen in Fig. 6(a), a plane shock wave generated at the impact surface propagates into the aluminum part and generates

**Fig. 5** Computational grid models of the projectile and container

a plane shock wave at the solid-liquid boundary inside the container. The shock waves in the solid aluminum propagate faster than those in the suspension liquid because the speed of sound in aluminum is about 3.4 times that in the liquid. The pressure disturbances generated by deformation of the free boundaries of the container catch up with the shock wave front propagating in the aluminum part and change the shape of the wave front. In Fig. 6(b), the diffracted shock wave in the aluminum propagates around the boundary of the liquid, while the shock wave in the suspension liquid maintains almost a thin plane shape. In the aluminum part, local areas of negative pressures are observed behind the diffracted shock wave and also near the impact surface. As shown in the suspension liquid of Fig. 6(c), weak pressure changes are observed in the path of the shock wave. These pressure perturbations in the suspension liquid are generated by the reflection of the diffracted shock waves in the aluminum part at the axis of symmetry. On the other hand, Figs. 6(b) and (c) show that the thin plane shock wave in the suspension liquid is propagating while decreasing in size. In Fig. 6(d), the plane shock wave in the suspension liquid arrives at the liquid-solid boundary, then is reflected and generates high pressure in the liquid. These computational results show that the complicated changes of pressure in the aluminum container result from the different characteristics of wave motions in the media of the solid aluminum and suspension liquid, and from the disturbances from the boundaries of the container. However, we can see that the fluctuations of the first shock pressure at the gauge position are generated by the plane shock wave propagating in the suspension liquid.

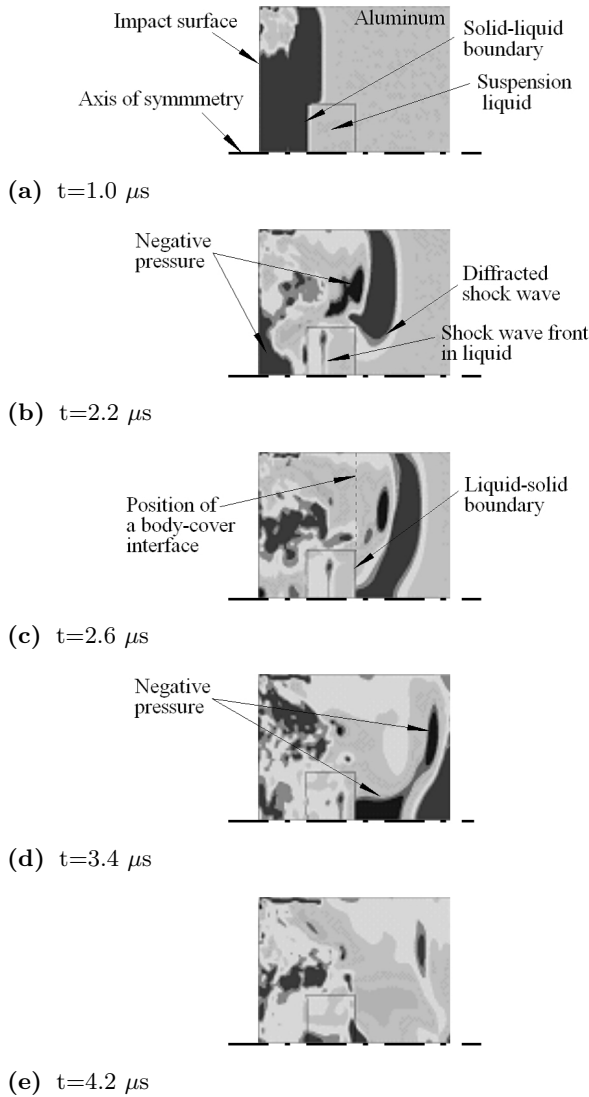


Fig. 6 Hydrodynamic pressure distributions in the container ($V_p = 96.2$ m/s)

Figure 7 shows a comparison between the experimental and the numerical pressure fluctuations at the impact velocity of 96.2 m/s. The bold and thin lines are the experimental and the numerical results, respectively. The time at the first pressure peak of the numerical curve is coincident with the experimental curve. In the experiment, the first strong spike-like pressure, which has a peak value of 239 MPa at about $4 \mu s$, is generated by the first direct reflection of the plane shock wave propagating in the suspension liquid at the gauge surface. The computational result indicates 222 MPa corresponds to the first experimental peak pressure. In the computational result, small positive and negative pressure fluctuations are shown just before the rise of the first strong shock wave. As mentioned above, these pressure fluctuations are caused by the high pressure generated by the reflection of the diffracted waves in the aluminum at the

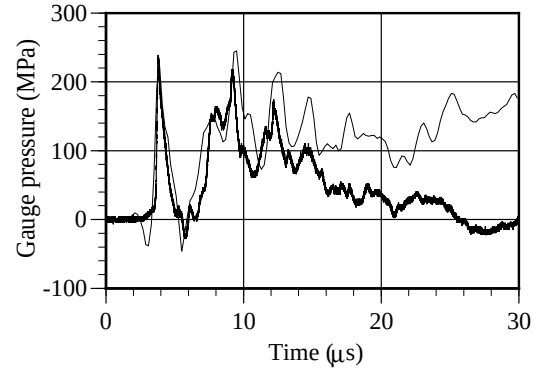


Fig. 7 Comparison between the experimental (bold line) and computational (thin line) pressure fluctuations ($V_p = 96.2$ m/s)

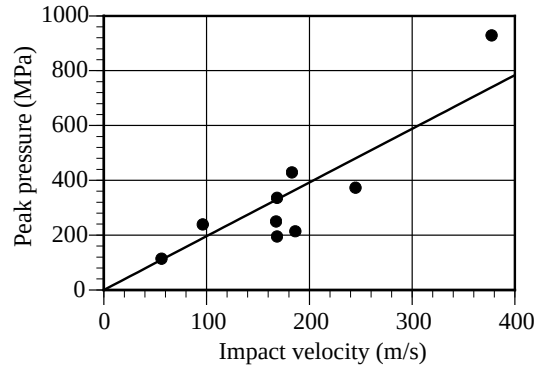


Fig. 8 Relation between the impact velocity and peak pressure obtained from the experiments

axis of symmetry. In the numerical simulation, the interface between the body and the cover of the aluminum container is not considered, so that the shock waves in the aluminum can propagate without being attenuated at the interface. On the other hand, in the experiment, we cannot ignore the influences of the body-cover interface on the strength of the shock waves. In addition, the glue used to stick the piezofilm gauge on the surface of the cover also attenuated the shock waves propagating from the aluminum cover into the suspension liquid. From the comparison of both the experimental and numerical pressure fluctuations after the first shock wave, it is shown that the pressure profiles agree with each other until about $10 \mu s$. But, after that, the experimental pressure is lower than that of the computational result, and the difference between them increases gradually. Leakage of pressure from the container may also occur in the experiment.

The abovementioned results indicate that the present numerical simulation can predict precisely the motion of the shock waves and the pressure fluctuations in the aluminum container until a plane shock wave propagates through the liquid and is reflected at the liquid-solid boundary.

4.2 Effect of shock pressures on the inactivation of the marine *Vibrio* sp.

Figure 8 shows the relation between the impact velocity and the first peak pressure. Using the present experimental method, we can get pressures of several hundred MPa at the peak of the shock waves in the suspension liquid sealed in the aluminum container. These pressures correspond with the impact velocities. That is, it is found that the peak pressure is proportional to the impact velocity. The solid line in Fig. 8 is the linear regression line and can be written as

$$P_g = 1.96 \times V_p, \quad (4)$$

where P_g (MPa) is the peak pressure of a shock wave reflected at the gauge surface, and V_p (m/s) is the impact velocity.

Figure 9 shows the relation between the number of viable cells and the impact velocity. The number of viable cells is represented by $\log(\text{CFU/ml})$, where CFU is the colony forming units [14]. The number of control cells, which is the initial number of viable cells before the shock event, was of the 10^8 order in CFU/ml. In this figure the number 0 on the ordinate axis represents complete inactivation. It is found that the number of viable cells decreases exponentially until the impact velocity of 115 m/s, and so we can have complete inactivation of the cells at velocities higher than 115 m/s. The pressure at this condition is estimated to be 200 MPa. These values are confirmed as the thresholds for the inactivation of the cells in this experiment.

4.3 Electron microscopy and protein analysis

An electron microscope (XL-30CP, FEI company) was used to observe the cell damage due to the shock wave pressures. Figures 10(a)-(c) show micrographs of the marine *Vibrio* sp. magnified at 10,000 times. As seen in Fig. 10(a), the configuration of the control cell is a spheroid, of which the average larger length is about $1 \mu\text{m}$. Figure 10(b) shows cells inactivated by exposure to a shock wave, and Fig. 10(c) shows cells under the osmotic pressure condition of NaCl (2 M) for 4 hours. All of the inactivated cells deformed by the shock waves resembled red blood cells, while the cells subjected to osmotic pressure were deformed irregularly. It is found that these two pressure conditions produced different deformations. These observations indicate that the shock wave pressures caused deformation of the cells, but we could not see that the shock pressures caused serious destruction of the cells.

Table 3 lists the mass of protein in the suspension liquid before and after the shock wave event. Before the shock wave event, the clear supernatant liquid of the suspension formed from centrifugation did not include any protein, and there was 0.70 ± 0.01 mg protein/ml in the

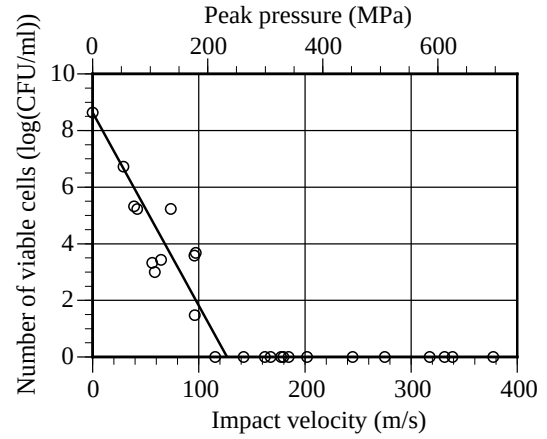
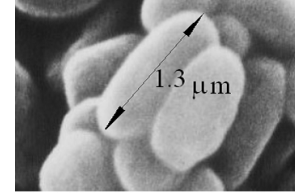
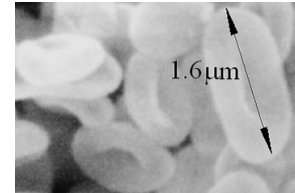


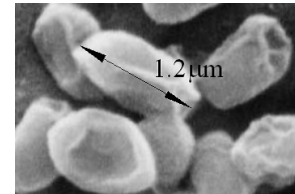
Fig. 9 Number of viable cells vs. the impact velocity and peak pressure. Zero on the ordinate axis indicates complete inactivation of the cells. CFU is the units of colony forming cells



(a) Control cells



(b) Shocked cells



(c) Cells under osmotic pressure

Fig. 10 Electron micrographs of the marine *Vibrio* sp.

sediment. After the shock wave event, the mass of protein increased to 0.07 ± 0.02 mg protein/ml in the clear supernatant liquid, and it decreased to 0.62 ± 0.02 mg protein/ml in the sediment. These results confirm that the mass of protein leakage was only about 11% of the total mass of the control cells. These facts suggest that the leakage of protein occurred at the outer membrane of the cells.

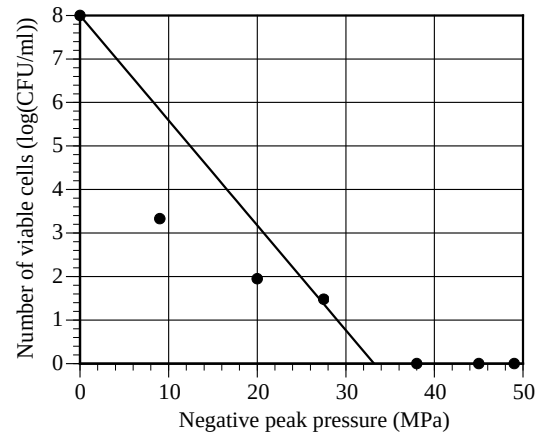
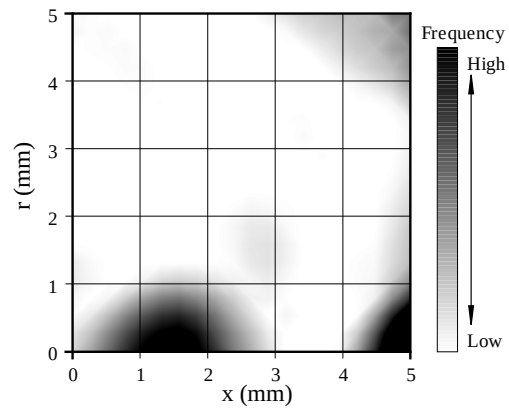
Table 3 Protein mass in the suspension liquid

	Before shock event (mg protein/ml)	After shock event (mg protein/ml)
Supernatant	—	0.07±0.02
Sediment	0.70±0.01	0.62±0.02

4.4 Relation between the negative pressures behind the shock waves and inactivation of the cells

The profiles of all the shock wave pressures indicated fundamentally the same characteristics in the experiments using the impact velocity of 56.1 to 377.4 m/s. Each pressure fluctuation had a strong spike-like pressure change, followed by a negative pressure change and then a pressure fluctuation of large wavelength. In the previous section, we discussed the relation between the peak pressure of the shock wave and the inactivation of the marine *Vibrio* sp. In addition, importance of the impulse of shock wave for fluorophore uptake into living cells has been reported by Kodama et al. [19]. We consider that the impulse of the first shock wave seems to be also related to the inactivation of cells. However, in general, considering the destruction of cells exposed to the shock waves, the behavior of negative pressures is primarily important. In much of the experimental data, the first spike-like shock wave, followed by the strong negative pressure change, was observed, and the inactivation of cells was relatively positive. Figure 11 shows the relation between the number of viable cells and the negative peak pressure behind the first shock wave. A decreasing number of viable cells is seen with the increase of negative pressure. The strong negative pressure produces not only a mechanical effect on the cells but also a chemical reaction. Additionally, the strong negative pressure change can generate cavitation bubbles in the container. In particular, the steep recovery from the negative peak pressure is considered to indicate the generation of cavitation bubbles in the suspension liquid. It is thought that the generation of cavitation bubbles may have a stimulating effect on the inactivation of the marine *Vibrio* sp. Kamath et al. [20] have carried out theoretical and numerical studies on the sonoluminescence mechanism. According to their paper, the dissociation of water vapor in oscillating cavitation bubbles resulted in the production of OH radicals, which are a kind of active oxygen. If OH radicals occurred in the suspension liquid due to the negative pressures behind the first spike-like shock wave, a chemical reaction would take place in the suspension liquid containing the bacteria, and the radicals would inactivate the bacteria efficiently.

In order to consider the possibility of the generation of cavitation bubbles that would produce OH radicals, the numerical result of the frequency of occurrence of negative pressure in the suspension liquid is shown in Fig. 12. This figure represents the upper half of the liq-

**Fig. 11** Relation between the number of viable cells and negative peak pressure behind a shock wave front**Fig. 12** Frequency distribution of the occurrence of negative pressure in the liquid, as obtained from the numerical calculation. x and r are the distances from the solid-liquid boundary and the center of the container, respectively

uid in the cross-section of the container, and the x axis corresponds to the axis of rotational symmetry. In this figure, it is found that the high-frequency areas are only near the axis of symmetry and the right boundary ($x=5$ mm). Therefore, local areas in the suspension liquid are only affected by the mechanical action due to the strong negative pressures. If OH radicals are produced by cavitation bubbles in the negative pressure areas, the action of oxidation would not affect all of the cells in the suspension volume. From the abovementioned examination, it is hard to explain that we can get the inactivation of the marine *Vibrio* sp. only by negative pressures, i.e., the positive pressures of the shock waves play an important role in the inactivation of the cells. In order to clarify the mechanism of the inactivation process of the cells, we need more experimental data to reasonably explain the relation between the shock wave profile and the inactivation of the marine *Vibrio* sp.

5 Conclusions

We carried out impact experiments using a gas gun and a numerical simulation by LS-DYNA in order to study the effect of shock wave pressures on the inactivation of the marine *Vibrio* sp. In the experiments, we obtained pressures of several hundred MPa in a target container without destruction of the container and without leakage of the suspension liquid. The numerical simulation, using the Johnson-Cook elastic-plastic model for the aluminum and the Tait equation for the suspension liquid, indicated that the behavior of the shock waves in the solid and liquid parts of the aluminum container could be reasonably predicted. However, in order to improve the precision of the simulation, the equation of state of the negative pressures in liquid needs to be considered. In the present experiments, it was found that the values of the gauge pressure of the shock waves required for complete inactivation of the marine *Vibrio* sp. were higher than 200 MPa in the aluminum container. In addition, electron microscopy and a protein analysis showed that the shock wave pressures did not destroy the bacteria; instead, the bacteria were deformed like the shape of red blood cells. The present results suggest that the mechanical and chemical effects caused by the spike-like positive pressure change and the following negative pressure fluctuation probably relate closely to the inactivation of the marine *Vibrio* sp. In order to clarify the mechanism of inactivation of the cells by shock wave pressures, the effect of the shock wave profiles on the inactivation of the cells needs to be investigated.

Acknowledgements A part of the present study has been supported by JSPS under the grant No. B-16360437, and C-18560768. The authors would like to express their thanks to Dr. T. Tsuda of CRC Solutions Inc. for his technical assistance in the LS-DYNA simulation, and Mr. H. Kawasaki, a graduate student at Kobe University, Graduate School of Science and Technology, for his assistance with the gas gun experiment and the numerical simulation.

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