On mechanism of non-heating sterilization using the underwater shock wave loading and gas formation

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ABSTRACT

In the field where the thermal sterilization can't be applied, the establishment of the sterilization technology with non-heating is strongly requested. The sterilization by pressurizing is one of the sterilization technology. Especially, the underwater shock wave causes scarcely heat in pressurizing because the pressurizing time is extremely short. That is, it is thought that the underwater shock wave enables non-heating sterilization that originates only in pressure. Hence, in this research, the underwater shock wave loading caused by explosive was used for non-heating sterilization.

Saccharomyces cerevisiae, one of the budding yeast was used for experiments. *S. cerevisiae* starts fermentation by feeding the glucose, and causes CO_2 within its body. There is the great density difference between cells of *S. cerevisiae* and the gas, hence, the acoustic impedance is different on the underwater shock wave transmission. Therefore, a strong reflected wave is caused on the boundary of the cell and the gas, and a remarkable expansion is caused. Fermented *S. cerevisiae* are sterilized by this phenomenon, and showed high sterilization rates. The sterilization rate by the underwater shock wave was low for not giving the glucose, that is, *S. cerevisiae* that had not fermented. The sterilization rate that had been done on three conditions was as follows in the order of higher. 1) Fermenting *S. cerevisiae,* high pressure. 2) Fermenting *S. cerevisiae*, low pressure. 3) Non-fermenting *S. cerevisiae,* high pressure.

The detonation fuse was used in this experiment. There was an interesting phenomenon, that is, the sterilization rate was high at the side of detonation beginning, and it was decreased toward the direction. It is

thought that this is related to a constant angle of the shock wave caused from the detonation fuse. A corresponding result to the phenomenon was gotten by the numerical analysis between the progress of the detonation and the change of pressure.

Keywords: Underwater shock wave; Non-heating sterilization; Numerical analysis

1. INTRODUCTION

In general, a typical sterilization technology is a heating sterilization. However, especially in the food sterilization, the following problems are pointed out about the heat-sterilization.

The first problem is a thermal alteration. The heat transformation of the protein is a nonreciprocal phenomenon and the heating cooking is the one to use this phenomenon. For example, the coagulation of the egg by heating is a typical thermal alteration. That is, a thermal alternation becomes a trouble for raw ingredients. In recent years, many kinds of variegation of gastronomic cultures are demanding a lot of raw materials. Pasteurization is used as the means for solving this problem.

The second problem is the ununiformity of the heat transmission. For instance, the food of the fine particle like flour contains a lot of air between the particle and the particle. In air, the heat transmission is very low with 4 kcal/(m^2·h·^{o}C). Therefore, only the part exposed to the heat source is sterilized, and the bactericidal effect doesn't appear easily on the inside of the fine particle food. The sterilization method that uses the gas such as ozone is used as the means for solving this problem.

In addition, the problem by thermotolerant bacterium, for example, *Alicyclobacillus acidoterrestris* occurs in the heating sterilization [1].

There are as mentioned above various problems in the heating sterilization method. And, neither the pasteurization nor the gas sterilization method obtain the position as a complete substitution means of the heating sterilization. Then, this research experimented on the sterilization using the high pressure generated by the underwater shock wave. Pressure physically acts on the vital activity. It is said that pressure necessary for being inert of the bacillus is several hundred MPa [2]. In the food sterilization technology using the high pressure, the electrical pulse [3] and high hydrostatic pressure treatment [4] are main currents. However, the pressure load of a multi frequency or a long time is required in these methods. In this research, the underwater shock wave for an extremely short time at just one time generated by explosives was tried for the sterilization.

2. SAMPLES

Saccharomyces cerevisiae was prepared as samples for the sterilization. *S. cerevisiae* is a typical budding yeast that increases by germination. It is known by generating alcohol by fermentation that obtains glucose, and used as a living thing of the model of the eucaryocyte. In general, it is known by the alias of "Yeast" when especially used to cook.

The scanning electron microscope (SEM) photograph of bacteria used by the actual experiment is shown in Figure 1. This photograph was taken after underwater shock wave loading. Dryness and electrically conductive are requested for observing the biological sample using SEM. Then, the pre-fixation, post-fixation, dehydration, and post-gold evaporation were processed according to the following. The purpose of pre-fixation and post-fixation is to prevent the sample change in shape caused by dehydration. The gas

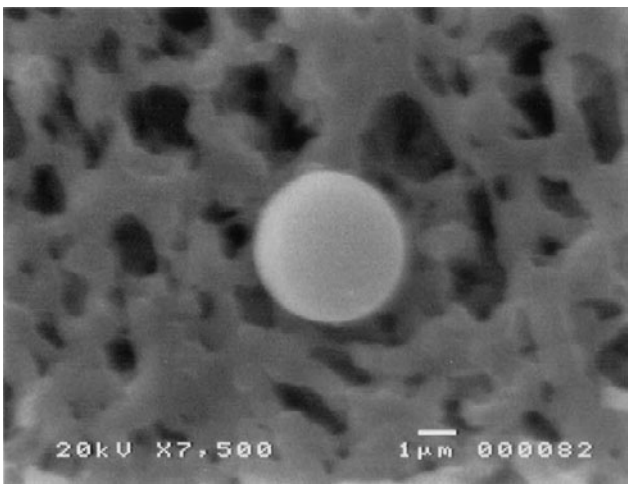


Figure 1 The scanning electron microscope photograph of *Saccharomyces cerevisiae* after underwater shockwave loading.

discharge from the sample observing SEM by dehydration is prevented. And, the sample obtains electrically conductive by gold evaporation.

2.5% glutaraldehyde fixation diluted with the phosphoric acid buffer solution was used for the pre-fixation of *S. cerevisiae*. The pre-fixation was processed for 1 hour and 20 minutes under the environment approximately 4 degrees C. It washed for 60 minutes with the phosphoric acid buffer solution after the pre-fixation, in addition, it soaked it to the phosphoric acid buffer solution during night. 1% osmium solution was used for the post-fixation. The fixed time is one hour. After fixation had been completed, it dehydrated by six stages with the ethanol from 50 to 100%. The sample had been coated (gold evaporation) according to the following conditions before taking a photograph. Au is used for the target, and the ionization current is direct current 10mA. The coating of the thickness of 300 angstroms was given by the electrical discharge of four minutes. The rough vacuum scanning electron microscope was used for taking a photograph, and the accelerating voltage was 20kV.

3. EXPERIMENTAL SET-UP

Figure 2 shows the experimental set up. The detonating fuse (made of The Japan Carlit Co., Ltd., detonation speed 6308m/s) was used for the shock wave source, and the electric detonator (made of Asahi-Kasei Industrial Co., Ltd., No.6) was used for detonation. After bacteria had been sealed up to prevent the contamination, bacteria were flatly arranged on the stainless plate. In addition, the silicon rubber seat of 3mm in thickness was set up for the protection of sealing up. Three detonating fuses were arranged and bundled, and the distance was taken and set from the sample. The length of two detonating fuses is 0.31m, and one detonating fuse with the electric detonator is set is 0.34m. As for the contained amount of the explosive, the Pentaerythritol Tetranitrate (PETN) is about 9.7g in total of three detonating fuses. The pressure of an underwater shock wave is decided according to the distance between the detonating fuse and the sample. For instance, The load underwater shockwave pressure is approximately 267MPa when there is a distance of 40mm from one detonating fuse [5].

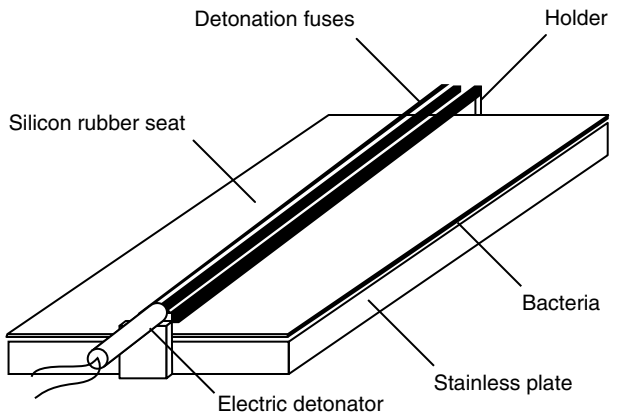


Figure 2 Experimental setup using three detonation fuses.

4. PROCESS

After the underwater shock wave loading, *S. cerevisiae* was incubated in the appropriate agar medium (culture media). The Potato Dextrose Agar "Nissui" was used for incubating. The temperature for the incubation was set to 37 degrees C. After the incubating of 48 hours, colonies caused on the agar medium were counted.

5. RESULTS

Experimental conditions are shown in Table 1. Experimental condition was divided to examine the influence of the glucose addition. *S. cerevisiae* to which the glucose was added fermented, and CO_2 was generated to the inside of the body. *S. cerevisiae* generated CO_2 in experimental conditions A and B. And it didn't generate in the experimental condition C.

The number of colonies caused on the culture media is shown in Figure 3. It is thought that one colony was generated from one bacterium individual. The untreated sample that is the control was sealed up as well as the treated sample, and not loaded by underwater shockwave. Samples 1-7 are numbers of colonies under experimental condition A in *S. cerevisiae* that fermented by adding the glucose and treated by the underwater shock wave of 350MPa. It shows that the colony generated in the sample that obviously did the underwater shock wave loading is few. Samples 8-13 are numbers of colonies under experimental condition B in *S. cerevisiae* that fermented by adding the glucose and treated by the underwater shock wave of 184MPa. Some colonies were generated from treated samples of experimental condition B. However, their numbers are few compared with colonies caused in control samples. Samples 14-20 are numbers of colonies under experimental condition C in *S. cerevisiae* that not-fermented and treated by the underwater shock wave of 308.5MPa. Colonies were generated from treated samples by underwater shock wave of experimental condition C. These numbers are more than in experimental condition A and B a lot.

To consider the experiment results, the sterilization rate was calculated by the following. The result is shown in Figure 4.

$$SR = 100 \times \left(1 - \frac{T}{C} \right)$$

Table 1 Conditions of experiments

	Condition A	Condition B	Condition C
Pressure (MPa)	350	184	308.5
Glucose addition	+	+	−

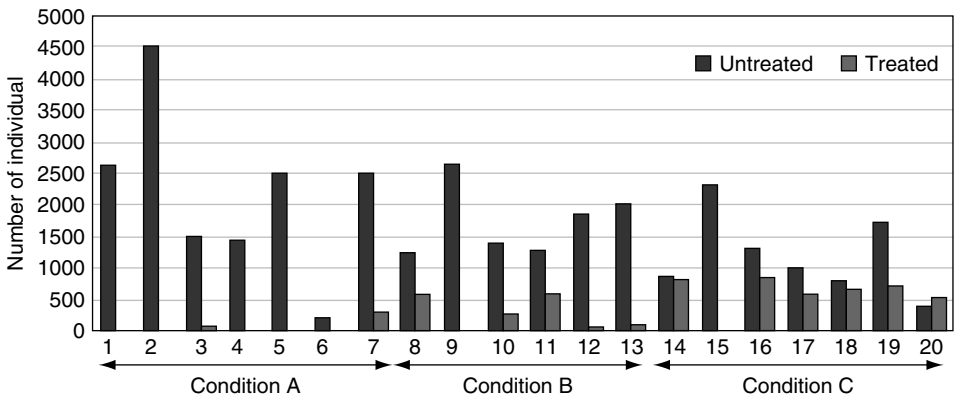


Figure 3 Number of treated and untreated individuals of *S. cerevisiae* after incubation. Condition A (Sample No. 1-7): 350MPa and glucose addition. Condition B (Sample No. 8-13): 184MPa and glucose addition. Condition C (Sample No. 14-20): 308.5MPa and glucose non-addition.

SR: The sterilization rate.

C: Number of colonies on the untreated sample.

T: Number of colonies on the sample treated by the underwater shockwave.

To consider the experiment results, the sterilization rate was calculated by the following. The result is shown in Figure 4. The sterilization rate in experimental conditions A (the fermentation condition and high pressure shock wave) is 87.2-99.87%, and the mean value is 96.67%. In experimental condition A, high and stabilized sterilization rates are seen. The sterilization rate in experimental conditions B (the fermentation condition and low pressure shock wave) is 88.14-99.73%, and the mean value is 85.55%. In experimental condition B, sterilization rates are not stabilized. The sterilization rate in experimental conditions C (the no-fermentation condition and high pressure shock wave) is 42.51-99.40%, and the mean value is 63.05%. In experimental condition C, the sterilization rate is approximately 60% excluding one exception, and these are lower than that of experimental condition B. These results suggest that the fermentation influence the sterilization rate more than the pressure of the shock wave.

Figure 5 shows the sterilization rate obtained from a past experiment, using the underwater shock wave loading and *S. cerevisiae* [6]. Experimental condition were the fermentation by the glucose addition and the underwater shock wave loading of 300MPa. In a word, it is similar to experimental condition A in this research. The explosion has progressed from 1 of the sample numbers toward 7. The graph of Figure 7 suggests a decrease of the sterilization rate that accompanies the progress of the explosion. The similar tendency is seen in experimental condition A of Figure 4, too. The numerical simulation was used to consider this tendency, based on the change in pressure by progress of the explosion.

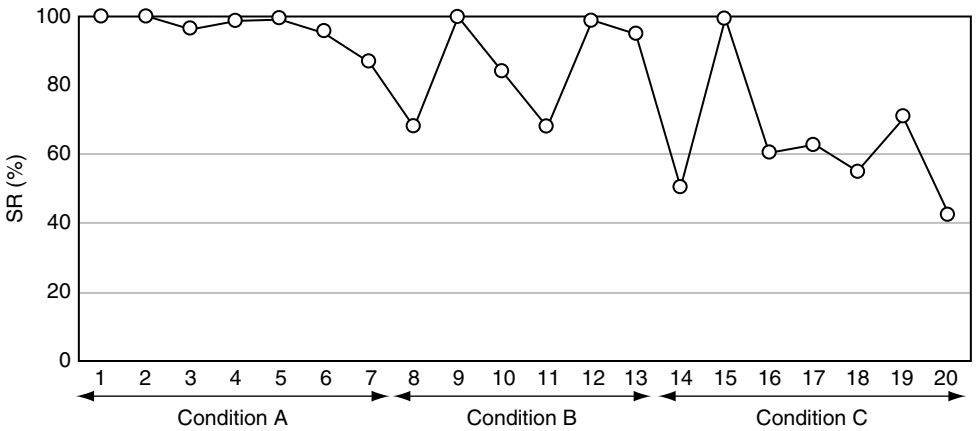


Figure 4 The sterilization rate of *S. cerevisiae* Condition A (Sample No. 1-7): 350MPa and glucose addition. Condition B (Sample No. 8-13): 184MPa and glucose addition. Condition C (Sample No. 14-20): 308.5MPa and glucose non-addition.

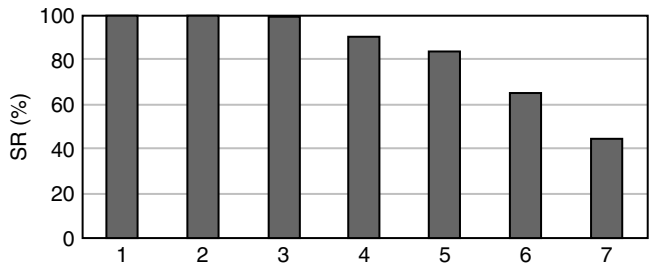


Figure 5 The sterilization rate of *S. cerevisiae*. The underwater shock wave treatment condition is 300MPa and glucose addition.

6. NUMERICAL SIMULATION

This analysis model is simulated with the multi-material formulation of LS-DYNA3D. The fluid is solved by using an Eulerian approach. The Eulerian formulation is used for the detonation product of explosive and water in this study.

The governing equations for the fluid formulation are given by:

(i) The equation of mass conservation

$$\frac{\partial \rho}{\partial t} = -\rho \cdot div(v) - v_i \frac{\partial \rho}{\partial x_i}$$

(ii) The equation of momentum conservation

$$\rho \frac{\partial v_i}{\partial t} = \sigma_{ij,j} - \rho \cdot v_i \frac{\partial v_i}{\partial x_j}$$

(iii) The equation of energy conservation

$$\rho \frac{\partial e}{\partial t} = \sigma_{ij} \cdot \varepsilon_{ij} - \rho \cdot v_i \frac{\partial e}{\partial x_j}$$

where ρ is the density, σ is the Cauchy stress, and v is the velocity of fluid.

Table 2 JWL parameter for detonating fuse

	A (GPa)	B (GPa)	R_1	R_2	ω
DF	452.35	8.85	5.485	1.425	0.28

Table 3 Mie-Grüneisen parameters for water and SUS304

	ρ_0 (kg/m^3)	c_0 (m/s)	s	Γ_0
Water	1000	1490	1.79	1.65

Jones-Wilkins-Lee (JWL) equation of state for detonation product gas of the detonating fuse and Mie-Grüneisen equation of state for water and SUS304 is used in the model, because a hydrodynamic material model requires an equation of state to define the pressure-volume relationship.

JWL equation is used for the equation of state for the detonating fuse. This equation is shown below (15) and the parameters are shown in Table 2.

$$P_{JWL} = A\left[1-\frac{\omega}{VR_1}\right]\exp(-R_1V)+B\left[1-\frac{\omega}{VR_2}\right]\exp(-R_2V)+\frac{\omega e}{V}$$

$V = \rho_0$ (Initial density of an explosive) /ρ (Density of detonation gas), P_{JWL} : Pressure, e : Specific internal energy, A,B,R_1,R_2,ω: JWL parameter.

The Grüneisen equation is used for the equation of state of water and SUS304. Equation (16) gives Mie-Grüneisen equation in which the higher degree values are not taken into account. The values of the parameters are shown in Table 3.

$$P=\frac{\rho_0 {c_0}^2\eta}{(1-s\eta)^2}\left[1-\frac{\Gamma_0\eta}{2}\right]+\Gamma_0\rho_0 e$$

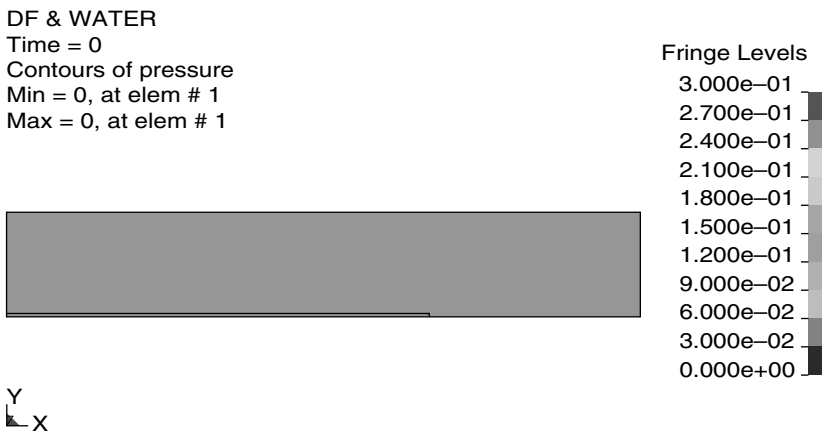
$\eta = 1-\rho_0$ (initial density of the medium) /ρ (density of the medium), P: Pressure, e: Specific internal energy, c_0,s: Constant of material, Γ_0: Grüneisen coefficient.

These results are shown in Figure 6 and Table 4. The pressure change to which the explosion progresses is shown, from top left to bottom right. The source of the shock wave is a base part. And the explosion progresses from the left right. The high pressure caused on the left side progresses toward right side and decreases.

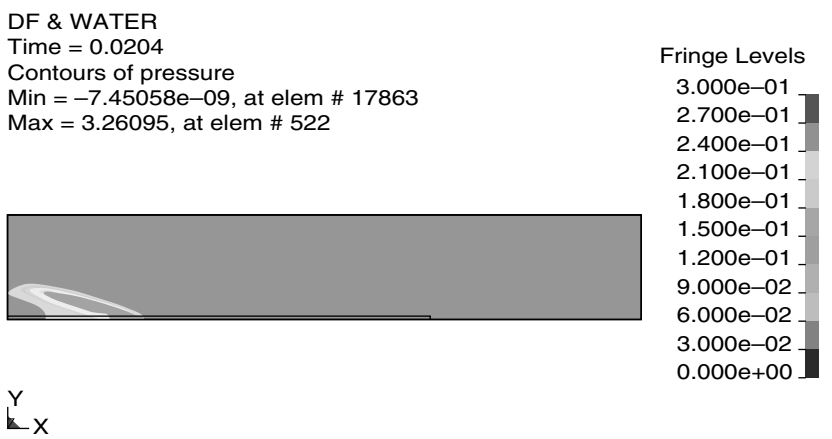
7. DISCUSSION

The sterilization rate in experimental conditions A shows a high sterilization rate with stability as shown in Figure 4. Experimental conditions B of the sterilization rate are more excellent though C of the pressure value is higher than that of experimental conditions B in experimental conditions B and C. *S. cerevisiae* has fermented by the glucose in experimental conditions B. and it has not fermented in experimental conditions C. It is thought that the result of the sterilization rate shows the influence by the existence of the bubble in fermentation in the

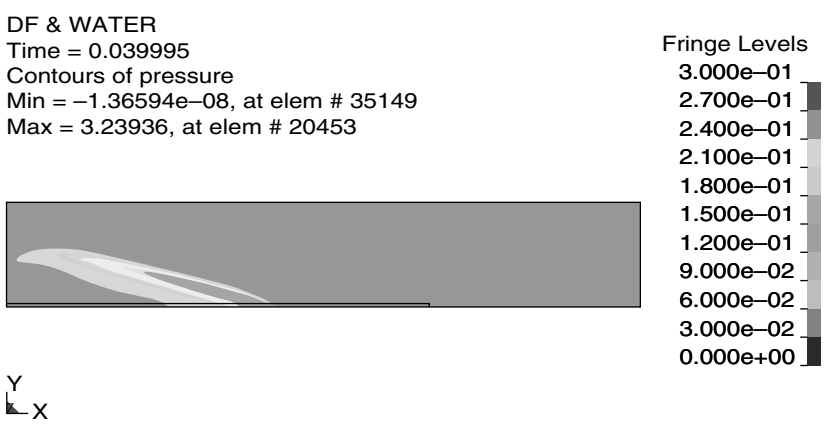
(a)


DF & WATER
Time = 0
Contours of pressure
Min = 0, at elem # 1
Max = 0, at elem # 1
Fringe Levels
3.000e–01
2.700e–01
2.400e–01
2.100e–01
1.800e–01
1.500e–01
1.200e–01
9.000e–02
6.000e–02
3.000e–02
0.000e+00
Y
X

(b)


DF & WATER
Time = 0.0204
Contours of pressure
Min = –7.45058e–09, at elem # 17863
Max = 3.26095, at elem # 522
Fringe Levels
3.000e–01
2.700e–01
2.400e–01
2.100e–01
1.800e–01
1.500e–01
1.200e–01
9.000e–02
6.000e–02
3.000e–02
0.000e+00
Y
X

(c)


DF & WATER
Time = 0.039995
Contours of pressure
Min = –1.36594e–08, at elem # 35149
Max = 3.23936, at elem # 20453
Fringe Levels
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2.400e–01
2.100e–01
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1.200e–01
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6.000e–02
3.000e–02
0.000e+00
Y
X

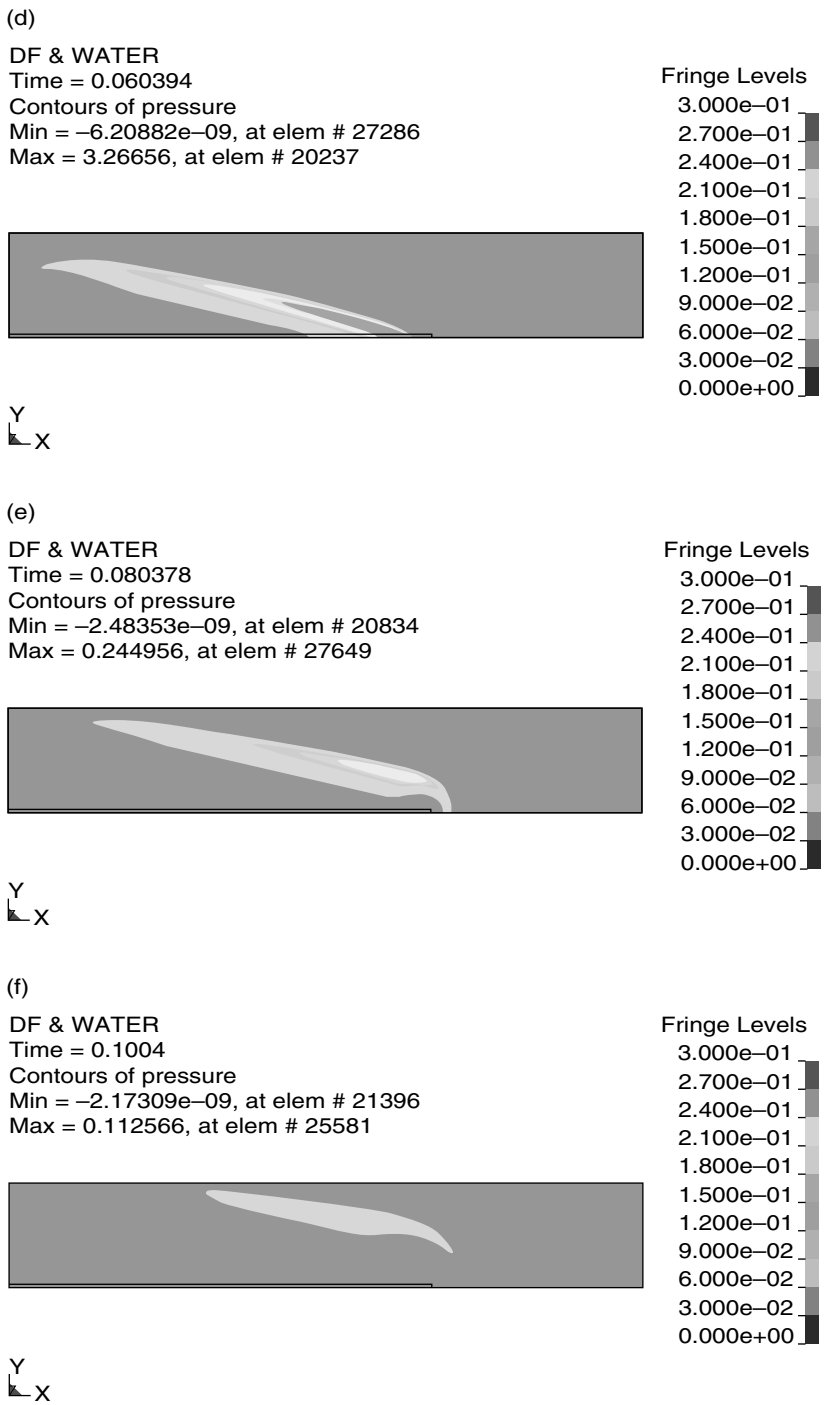

Figure 6 Numerical simulation results of the change in pressure by the progress of detonation. The pressure change to which the explosion progresses is shown, from top left to bottom right.

Table 4 Numerical simulation results of the change in pressure by the progress of detonation

		x-direction (mm)								
		0	50	100	150	200	250	300	350	400
y-direction (mm)	15	44	438	443	442	442	442	442	442	228
	20	24	368	377	377	377	377	377	376	192
	25	16	313	329	329	329	329	329	328	162
	30	8	269	290	290	290	290	290	289	138
	40	5	227	256	256	256	256	255	255	115
	80	3	25	61	75	77	76	75	63	13

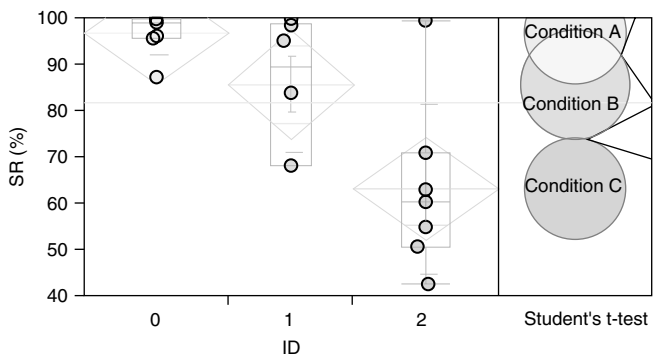

Figure 7 The one-way analysis of variance result of the sterilization rate (*S. cerevisiae*) and comparative circle by Student's t-test. Condition A: 350MPa and glucose addition. Condition B: 184MPa and glucose addition. Condition C: 308.5MPa and glucose non-addition.

bactericidal effect, that is, the bacillus inside of the body. To show it, the sterilization rate was analyzed by Student 's t-test. The one-way analysis of variance result of the sterilization rate and comparative circle by Student's t-test are shown in Figure 7. Statistics and analysis software JMP IN 5.1.2 (SAS Institute Japan Co.Ltd.) was used for the calculation.

A significant difference is not confirmed from the crossing angle of comparative circle of experimental conditions A and B because it is larger than that of 90 degrees. On the other hand, comparative circles of experimental conditions A and C do not intersect mutually. In addition, the crossing angle of each experimental conditions B and C comparative circle is smaller than that of 90 degrees. Experimental conditions are divided from these into C with the group of A and B. It is thought that these originate in the presence of the addition of the glucose, that is, fermentation.

It was clarified that the fermentation phenomenon influenced the sterilization by the underwater shock wave loading from the pressure value from the experiment result. On the other hand, a little change in pressure influences the sterilization rate in the fermented *S.cerevisiae*. These changes are requested by the math calculation, therefore it has been understood that the change of the pressure value is very minute.

8. CONCLUSION

The underwater shock wave loading shows a remarkable sterilization effect to fermented *S. cerevisiae*. The sterilization rate changes when the fermentation of *S. cerevisiae* is the same, by a little change of the pressure value of an underwater shock wave loading. That became clear by the numerical simulation. It will be necessary to investigate the relation between a more detailed fermentation condition and the pressure value in the future.

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