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INACTIVATION EFFECTS OF NEGATIVE PRESSURES BY A METAL BERTHELOT TECHNIQUE ON BACTERIA SOLUTIONS

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Abstract

Bacterial solutions are anticipated to be inactivated under absolute liquid negative pressures much lower in magnitude than positive ones. The pressures, however, have been hard to be produced by experiments because liquids form cavities easily through heterogeneous nucleation. To investigate the anticipation, solutions including two kinds of bacteria, namely Bacillus subtilis and Escherichia coli, were exposed to negative pressures repeatedly by a metal Berthelot tube which was designed newly. Then, numbers of colonies in bacteria which were cultured by a paper strip method and an agar dilute plate one were counted. Numbers of colonies which underwent negative pressures were less than those for non-treatment, and reduction rates of colonies increased with numbers of repetition. It was thought that inactivation effects of negative pressure on bacteria were due to destabilization of kinds of protein constituting the bacteria. Inactivation effects of negative pressure on bacteria will contribute to development of apparatus using ultrasound radiation by considering not only cavitation but also negative pressures without no chemical compound.

Keywords

Negative Pressure, Cavitation, Berthelot Method, Inactivation of Bacteria

1. Introduction

1.1 Literature Review

When a liquid is expanded isothermally, the liquid pressure decreases and can become negative because average distances between molecules comprising the liquid are longer than those at equilibrium. The liquid is in a stretched state which is thermodynamically metastable. Therefore, the liquid tends to cavitate through heterogeneous nucleation so that the liquid is in a co-existing state with its vapor. This phenomenon is called cavitation, which has made it difficult to generate negative pressure even now. Hence, studies on liquids' negative pressure have been much less than those on positive pressures.

Under such situation, studies have progressed little by little because liquids' negative pressure has been of basic importance in many fields of applied physics and engineering as mentioned in following examples. In studies on environmental stress cracking of polymers, which is a kind of serious delayed destruction, it has been insisted that the cracking is caused by increases in mutual solubilities between polymers and liquids as their non-solvents under dilative stresses which are equivalent to liquids' negative pressure (Okamoto & Ohde, 1986). In studies on phase diagrams of water including metastable states, it has been proposed that the diagrams elucidate the reasons for anomalous properties of liquid water by theoretical conjecture (Speedy, 1982) and molecular dynamic simulation (Chitnelawong, et al., 2019). In

studies on cavitation which is of relevant in the performance of hydraulic machineries or ultrasonic apparatus, it has been thought that the reason why tiny bubbles as cavitation nuclei can exist in liquids without disappearance due to dissolution to the liquids or buoyancy can be revealed by measuring negative pressure because negative pressures can be high in magnitude if cavitation nuclei are isolated and is removed.

Pressure and temperature are thermodynamic variables. Any material is stable or metastable under a given P-T condition. The world consists of soft materials like organics and polymers in addition to hard materials like metals and ceramics. Pressure dependences of soft materials should be much stronger than those of hard materials. Therefore, properties of soft materials under negative pressures would be more changeable than one can expect. It is significant to find a way to study experimentally the still inaccessible negative pressure world of those soft materials (Hiro, 2008).

Hence, a plan for finding a means for raising and maintaining negative pressures in liquids and measuring thermodynamic properties of liquids including polymer solutions under static negative pressures was started (Ohde, et al., 2001).

As a result of trial and error, metal tube Berthelot techniques for generation of -20 MPa order of negative pressure in liquids were established (Hiro, et al., 2019; Hiro, et al., 2003). Furthermore, by using the techniques, liquids' properties under negative pressure were feasible to be studied in temperatures of maximum densities for water (Hiro, et al., 2014), and phase diagrams including negative pressure regions for thermotropic liquid crystals (Ohde, et al., 2008).

A stability pressure-temperature diagram in a biological system has been drawn for a kind of bacteria, Escherichia Coli (Smeller, 2002), as shown in Figure 1. The diagram which seems to be elliptic partly for a lack of both negative pressure and subzero temperature regions insists that the bacteria are not lively away from the elliptical region so that the bacteria reduce numerically by two orders of magnitude within 5 min. The elliptical boundaries may be to be reached in a negative pressure region more easily than a positive one (Imre, 2002).

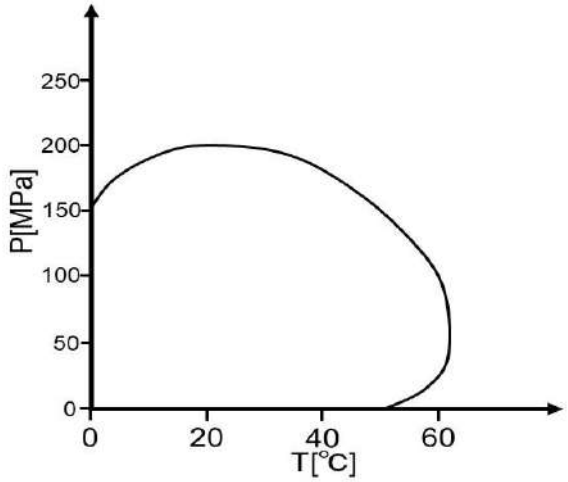

Figure 1: *Diagram of Escherichia Coli*

(Source: Smeller, 2002)

1.2 Research Issues

1.2.1 Problem identification

To authors' knowledge, no studies on inactivation of bacteria in negative pressure regions have been carried out.

1.2.2 Hypotheses formulation

Bacterial solutions are anticipated to be inactivated under absolute liquid negative pressures much lower in magnitude than positive ones. If the inactivation effects are confirmed, negative pressures will contribute to development of inactivation apparatus using cavitation such as ultrasound radiation without any chemical compounds because of not conventional effects of collapses of cavitation bubbles but new ones of negative pressures.

1.2.3 Research objectives

In order to investigate inactivation effects of liquid negative pressures, solutions including two kinds of bacteria, namely Bacillus subtilis and Escherichia coli, underwent negative pressures as a sample liquid in the metal Berthelot tube technique, and bacteria colonies which were cultured by a paper strip method and by an agar dilute one were counted.

2. Methodology

Berthelot methods using metal tubes can produce negative pressures statically through repetition of initial heating and subsequent cooling processes of sample liquids sealed in sample chambers in solid metal tubes over temperature ranges (Ohde, et al., 2008). Figure 2 (a) shows a pressure versus temperature graph of a liquid, while Figure 2 (b) depicts the liquid sealed in a cylindrical chamber in a top view. Since the liquid has a coefficient of thermal expansion higher than the solid, the initial heating process causes gases, which are composed of air and the liquid vapor remaining in the chamber as shown at the point G in each figure, to be forced from the liquid so that the chamber is filled with the liquid completely at a temperature T_f as shown at the point B in each figure and it's pressure increases steeply to the positive point C as shown in each figure (Trevena, 1987). The liquid forces the chamber wall to be expanded at the point C. In the next cooling process, the liquid adheres to walls of the chamber and keeps on to fill itself at temperatures below T_f as shown in the point D in each figure. The liquid forces the chamber wall to be contracted as shown in the point D. At a lower temperature T_b, the liquid breaks suddenly at the point E in each figure, and cavitation bubbles

appear with an impulsive increase in pressure as shown in the point F in each figure. In the next re-heating process, the bubbles are dissolved slowly with gradual increase in pressure until they disappear at the point B. By a further heating, the liquid pressure increases steeply to the point C. The liquid pressure is positive and negative above and below the T_f, respectively, and the alternative temperature process around the T_f, is called temperature cycle.

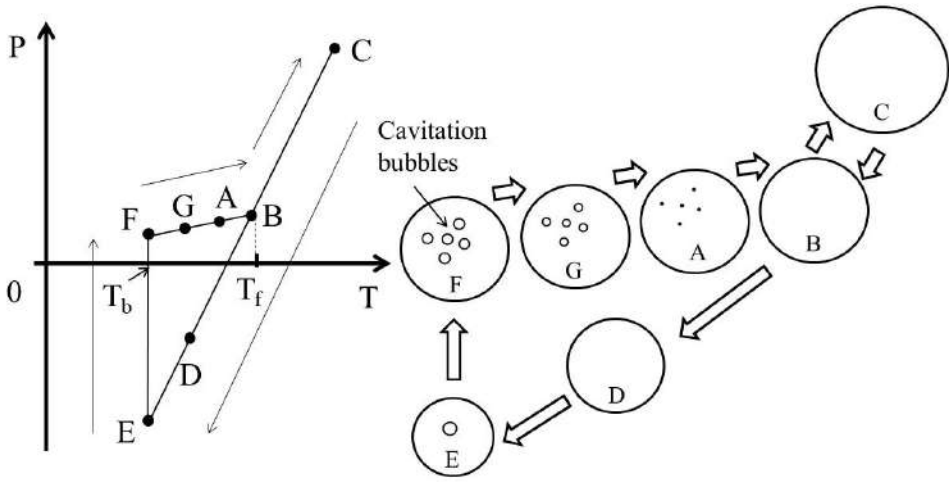

Figure 2: *Berthelot Tube; (A) A Pressure Versus Temperature Graph (B) A Liquid in The Tube*

(Source: Hiro, 2022)

In this study, prior to the check of inactivation effects for Bacillus subtilis, a solution including at least another kind of bacteria, that is, Escherichia coli, withstood negative pressures and the effects was investigated by a paper strip method (SUN CHEMICAL CO., LTD. No.1). The reason for the pre-check was that, to authors' knowledge, as the inactivation effects of negative pressures had not been reported experimentally, it was useful to get some information about results of promising Escherichia coli by a rough but time-saving method rather than an exact but time-consuming one.

In the preliminary test, a mixture of 90 ml distilled water and 10 g minced pork was used as the solution including at least Escherichia coli according to the paper supplier's advice.

Since a sample volume required for checking effects of negative pressures was at least 1000 mm^3, a newly developed metal Berthelot tube was designed. Figure 3 shows the new metal Berthelot tube. The Berthelot tube consisted of a screw, a brass socket, a rock nut, a brass ball and a strain gauge pressure transducer which had a chamber of ca. 1500 mm^3. The parts were made of type 630 stainless steel except for the ball and the socket. The screw had two grooves on threads parallel to an axis of the screw. A voltage signal of the transducer of was amplified by ca. 1250 times.

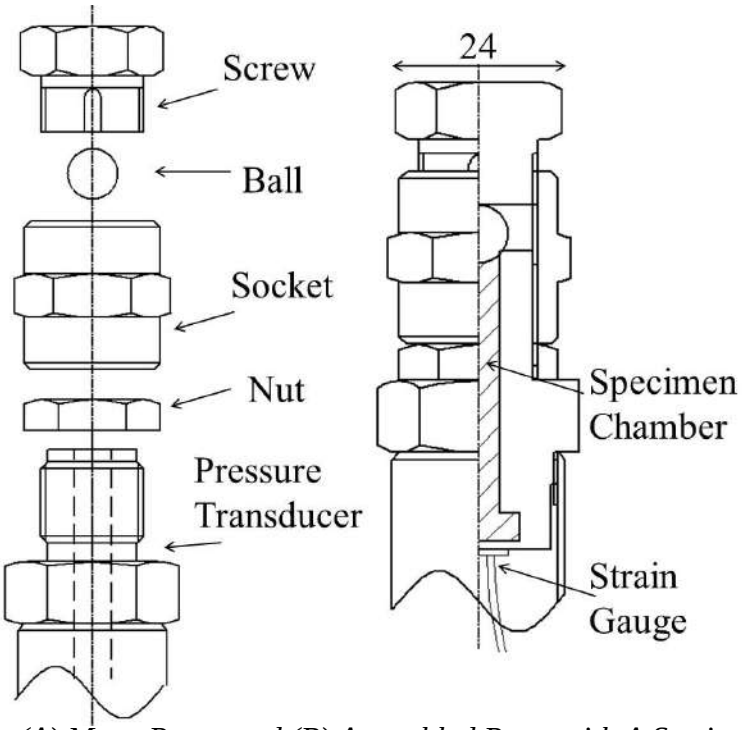

Figure 3: *Tube (A) Many Parts, and (B) Assembled Parts with A Sectional View (Source: Hiro, 2022)*

Sealing operation was done as follows: all parts were immersed in ethanol to be sterilized and then were dried sufficiently. The socket and the nut were attached to the transducer, and then, a bacterial solution as a sample liquid was poured into the chamber with a pre-sterilized micro-pipet of 1000 mm^3 up to the middle of the socket; the ball was dipped into the solution in the socket for sealing. Sealing was accompanied by pressing the ball under the solution. T_f was adjusted at ca. 50 °C by fastening the screw mildly at ca. 55 °C with a torque wrench by ca. 5 Nm. The tube had been fastened with the screw mildly was immersed in a hot bath of ca. 55 °C. After ca. 120 seconds taken to be almost equal for a temperature of the liquid in the tube to that of the bath, the tube was picked up from the bath, was fastened with the wrench by ca. 20 Nm, and was immersed again in the bath. As a result of the severely fastening, a voltage signal of the transducer increased to a level and were kept to the level. If the signal did not increase or increased to a level and decreased, it was judged that the sealing was insufficient for leakage from tube and the sealing operation was tried again.

If the sealing operation was successful, the tube was immersed in a hot bath of ca. 55 °C, and temperature cycles were repeated automatically as shown in Figure 5 (Hiro, et al., 2003). Voltage signals of the transducer of the tube were converted to digital ones with an analogue to digital converter in a programmable logic controller (PLC). According to the digital signals, the PLC controlled motions of a pulse motor which carried on temperature

cycles and recorded digitals signals which were equivalent to negative pressures at cycles. A pressure in the tube in the hot bath rose, and the PLC held the tube in the bath during a constant time. Then, it emitted pulses to the motor to immerse into a cool bath of ca. 10 °C.

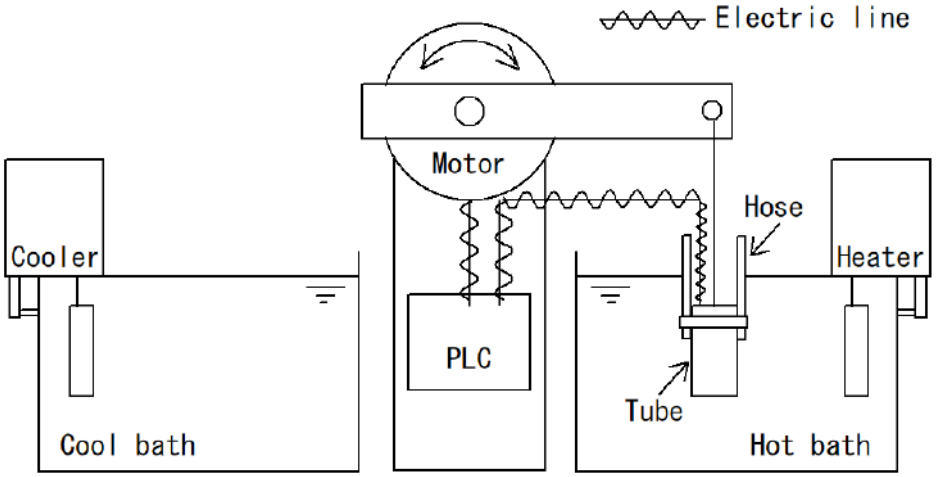

Figure 4: *Experimental Apparatus for Repetition of Temperature Cycles (Source: Hiro, 2003)*

As temperatures decreased, negative pressures built up and cavitation events occurred. Just after the cavitation events, the signals jumped up suddenly. The PLC subtracted a current signal at a cycle from that at the previous sampling time of ca. 0.1 ms. When the difference was negative over a definite period, it regarded the difference as a result of cavitation and returned the tube to the hot bath for the next cycle. In this way, the PLC repeated temperature cycles. A period for a temperature cycle took ca. 6 min.

After a definite number of temperature cycles were carried out, the repetition was stopped, and the tube was picked out of the bath. Compressed air was sent to a space remaining an amount of bath water inside the socket through one groove to drain off the water completely from the other. Then, the screw and the socket were unfastened in this order, and the ball was removed carefully from the top edge of the transducer. The sample solution was extracted carefully with a pre-sterilized micro-pipet of 0.5 ml.

Then, the extracted solution was checked about inactivation effects by a paper strip method for Escherichia coli. The paper strip was wetted uniformly with the solution, was enclosed in a plastic bag in which air had included as little as possible, and was put in an incubator for 0.5 day at 37 °C. Then, colonies on the paper were counted visually. For comparison, another solution which had not undergone temperature cycles and had kept in a bath of 40 °C for periods corresponding to relevant cycles was checked similarly. As it was

indicated that the Escherichia coli might be less even by leaving it alone, the solution was kept in the bath.

Another bacterial solution including Bacillus subtilis subsp. Spizizenii (JCM 2499, RIKEN) was prepared as a sample liquid. According to the supplier's manual, the solution included 2.5 % nutrient broth No.2 (Kanto Kagaku Co.).

In a similar way with the solution of Escherichia coli, the solution of Bacillus subtilis was sealed in the Berthelot tube, underwent definite temperature cycles, and then, was extracted carefully with a pre-sterilized micro-pipet of 0.5 ml.

The extracted solution was checked about inactivation effects by an agar dilution method because Bacillus subtilis was gram-positive bacteria, causing detection of the strip to be impossible. The solution was diluted by water of 4.5 ml. The solution of 10^{-1} in dilution rate was extracted with another micro-pipet of 0.5 ml and diluted again by another water of 4.5 ml. By a similar procedure, bacteria in solutions from 10^{-3} to 10^{-9} in dilution rates were cultured on agar plates for 2 days at 37 °C in an incubator. For comparison, another solution which had not undergone temperature cycles was checked similarly.

3. Results and Discussion

Figure 5 shows a trend in negative pressures for 20 temperature cycles for Escherichia coli solution. Negative pressures from ca. 2.1 MPa to ca. 6.9 MPa in magnitude were measured. The average and the standard deviation were ca. 4.2 MPa and ca. 1.1 MPa, respectively. Previously, sample liquids of Berthelot tube had undergone boiling and ultrasonic radiation to purge gases in the liquids for generation of negative pressures (Ohde, et al., 1989). In this study, it was found that the bacterial solution which had not experienced the treatments could generate the negative pressures higher than ca. 2.1 MPa in magnitude.

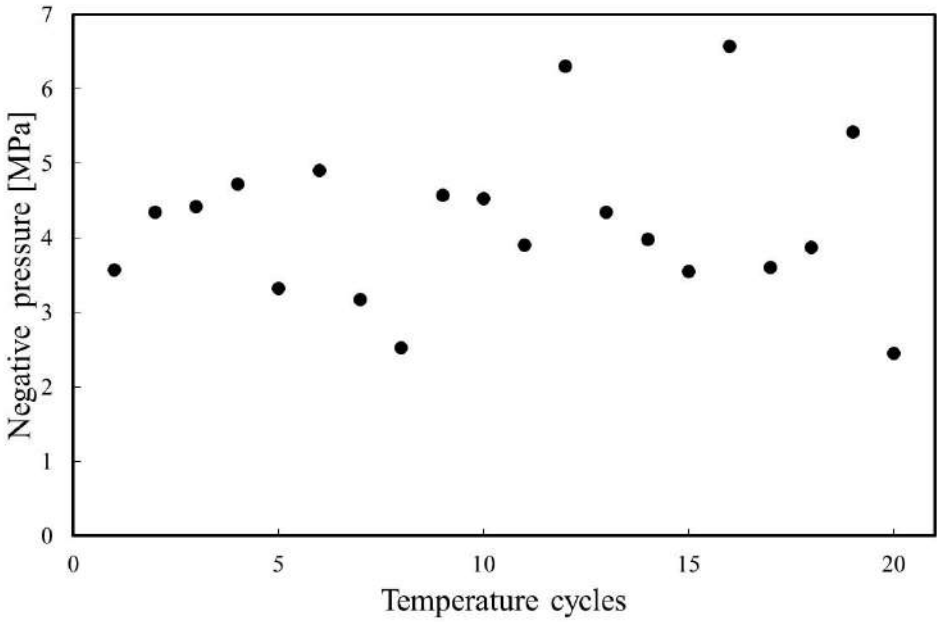

Figure 5: *Trend in Negative Pressures for Escherichia Coli Solution*

(Source: Self)

Figure 6 shows typical colonies on a paper strip which underwent temperature cycles. The colonies were counted as red points visually.

Figure 6: *Typical Colonies on A Paper Strip Which Underwent Temperature Cycles*

(Source: Self)

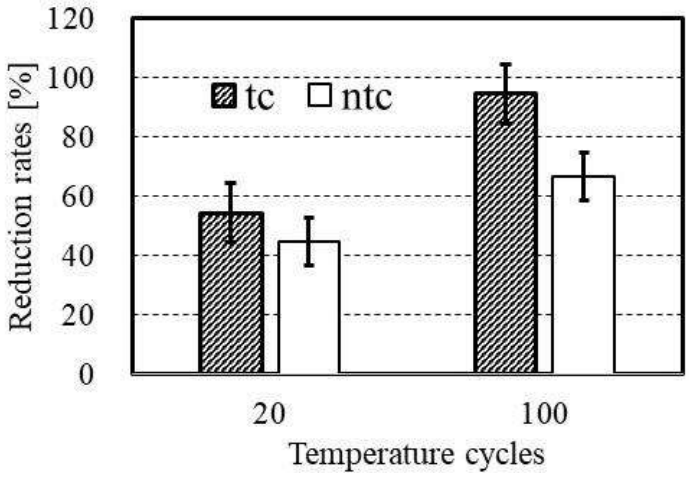

Figure 7: *Relation Between Reduction Rates and Temperature Cycles for Escherichia Coli*

(Source: Hiro, 2003)

Figure 7 shows reduction rates of colonies with temperature cycles. For comparison. reduction rates for solutions that underwent no temperature cycles and were kept in a bath of 40 °C for periods corresponding to relevant cycles were shown together in the figure, denoted as ntc. A reduction rate (R) was calculated by following equation;

$$R = \frac{(N_0 - N_C)}{N_0} \times 100$$

where:

N_0, N_C – numbers of colonies with no and definite cycles

Reduction rates for solutions which underwent temperature cycles were higher than those for no temperature cycles. Furthermore, the rates for solutions that underwent temperature cycles increased with the cycles more steeply than those for no temperature cycles. Thus, inactivation effects of negative pressures for solutions including at least Escherichia coli were confirmed by the paper strip method.

Since inactivation effects for the Escherichia coli. solutions were confirmed by the time-saving method, those for the Bacillus subtilus solutions were tested. In Figure 8, the

reduction rates attained nearly 90 % within 100 cycles, the Bacillus solutions underwent less temperature cycles in numbers, that is 13, 15, 20, and 30 cycles.

Figure 8 shows a trend in negative pressures for a solution including Bacillus subtilis. Negative pressures were from ca. 3.0 MPa to ca. 6.9 MPa in magnitude. The average and the standard deviation were ca. 5.1 MPa and ca. 1.1 MPa, respectively. Averages and standard deviations for different temperature cycles were almost the same values each other. Similarly in a case of Escherichia coli solutions, it was noted that the bacterial solutions which had not experienced boiling and ultrasonic radiation for purging any gases in the solutions could generate negative pressures.

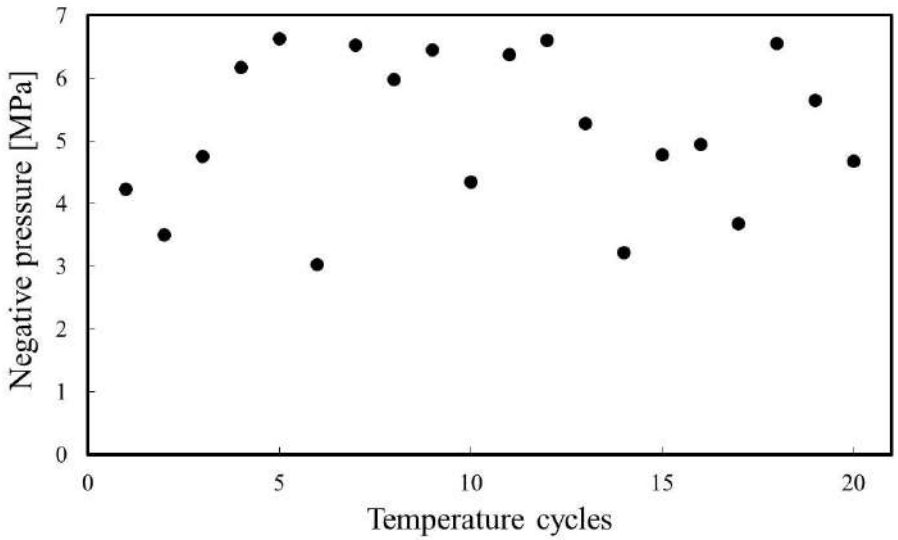

Figure 8: *Trend in Negative Pressures for Bacillus Subtilis*

(Source: Self)

Figure 9 show a typical photograph by an agar dilution plate method. In order to count colonies, they were marked with a black pen. Total numbers of colonies were calculated by multiplying numbers of colonies in the plate by an inverse of a dilution rate for the plate.

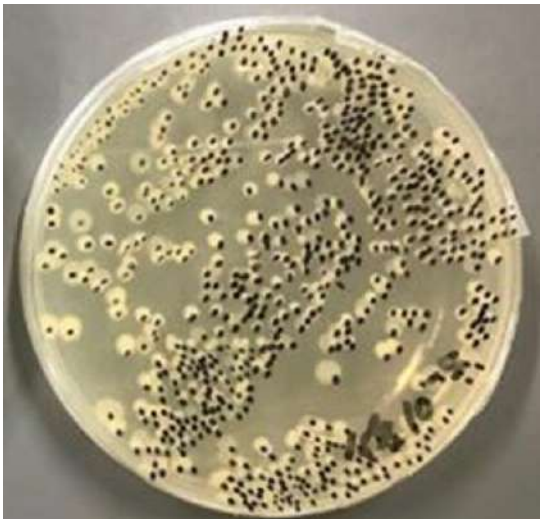

Figure 9: *Typical Photograph by An Agar Dilution Method*

(Source: Self)

For Bacillus subtilis, bacteria in solutions from 10^{-3} to 10^{-9} in dilution rates were cultured on agar plates for 2 days at 37 °C in an incubator, and colonies were counted visually with the pen. In some solutions, colonies were not able to be counted due to too large or too small numbers of them. Authors attributed these to incomplete dilutions of the solutions, and reduction rates were not calculated for them.

Figure 10 shows reduction rates for different temperature cycles. A reduction rate (R) was the same as that for Figure 7.

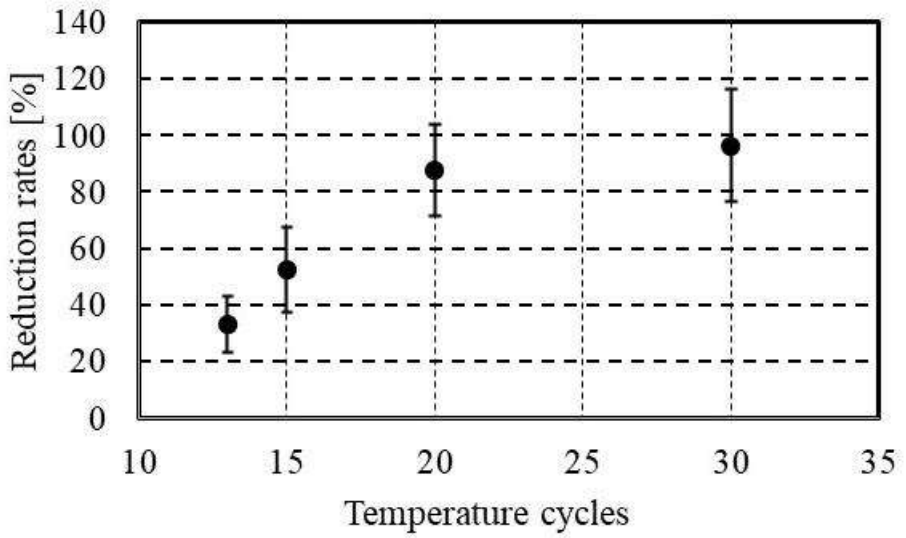

Figure 10: *Relation Between Reduction Rates and Numbers of Temperature Cycles*

(Source: Self)

Reduction rates increased with cycles as well as Figure 8 and attained nearly 90 % within 30 cycles. For two kinds of bacteria, namely Escherichia coli and Bacillus subtilis, the Berthelot technique generated negative pressures of at the highest 7.0 MPa with temperature cycles. According to Figure 1, the elliptical boundaries seem be higher in magnitude than the only 7.0 MPa. However, the figure insists a trend theoretically that the boundaries in a negative pressure region are much more attainable than those in a positive pressure one. The experimental 7.0 MPa is consistent with the theoretical trend.

Repetition of cycles was required for inactivation of bacteria. Authors estimated that it took ca. 20 s for the solutions to withstand negative pressures in one cycle. It is thought that, the higher temperature and the higher pressure in magnitude the bacteria solutions experience, the shorter a period for inactivation is. The estimated short period under the only low pressure

was insufficient to inactivate bacteria in the solutions perfectly for one cycle. It is though that repetition of cycles was needed.

By repeating cycles, cavitation events were also repeated. There have been many studies on removal of bacteria by cavitation events (Sarc, et al., 2019). They have insisted that bubbles' collapses occurring during extremely short periods yield emissions of shock waves or micro jets, causing the bacteria to be removed. According to results of visual observation on bubbles' behavior in a metal Berthelot tube built in a sapphire window, cavitation bubbles became small in size gradually by heating the tube with gradual increase in pressure signals, disappeared completely by further heating with steep one. In the present Berthelot tube, it seemed plausible that bubbles behavior were similar to those of the sapphire tube. It was found that a period from occurrence of cavitation bubbles to dissolution of the bubbles into the liquid took ca. 10 sec; the dissolution was very slow in comparison with their bubbles' collapses. Therefore, such slow bubbles' dissolution would not remove bacteria.

In general, bacteria are comprosed on many kinds of protein. It was reported that some kinds of pritein, that is, ubiquitin and myogrobin, destabilized under negative pressures which were much lower in magnitude than positive pressures (Larios & Gruebele, 2010; Smeller, 2002). It seems that inactivation effects of negative pressure on bacteria were due to destabilization of kinds of protein constituting the bacteria.

The sample volume of the tube was only ca. 1500 mm^3. Metal Berthelot tubes having larger sample volumes will be time-consuming to make negative pressures high for removal of larger amounts of cavitation nuclei and, therefore be difficult in direct use as any sterilization apparatus. However, in a kind of apparatus using ultrasound radiation, it is calculated that negative pressures higher than 20 MPa in magnitude are yielded for short periods but repeatedly (Tamura, et al., 2022). Such apparatus will be useful as a new means for inactivation of bacteria without any chemical compounds in future.

4. Conclusions

To investigate effects of negative pressures on bacteria, solutions of two kinds of bacteria, namely Bacillus subtilis and Escherichia coli were exposed to negative pressures repeatedly by using a metal Berthelot tube. Numbers of colonies of bacteria which were cultured by a paper strip method for Escherichia coli and an agar dilute plate one for Bacillus subtilis were counted. The colonies made from solutions had experienced negative pressures were less than those had not experienced, and furthermore, reduction rates of bacteria became

high with temperature cycles. Inactivation effects of negative pressure on bacteria were confirmed.

4.1. Research Limitations

The sample volume of the tube was only ca. 1500 mm^3. Metal Berthelot tubes having larger sample volumes will be time-consuming to make negative pressures high for removal of larger amounts of cavitation nuclei and, therefore be difficult in direct use as any sterilization apparatus. Accordingly, apparatus using ultrasound radiation that can generate negative pressures higher than 20 MPa in magnitude for short periods repeatedly will be useful as a new means for inactivation of bacteria without any chemical compounds in future.

4.2. Scope of Future Research

However, it has been reported that some apparatus using ultrasound radiation can generate negative pressures higher than 20 MPa or almost equal to 14 MPa in magnitude for short periods repeatedly. Such apparutus will be useful as a new means for inactivation of bacteria without any chemical compounds in future.

5. Acknowledgements

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REFERENCES

Chitnelawong P., Sciortino F., and Poole P., H. (2019). The stability-limit conjecture revisited. The Journal of Chemical Physics 150. https://doi.org/10.1063/1.5100129

Hiro K. (2008). Metal tube Berthelot techniques for generation of -20 MPa order of negative pressure in liquids. Nagoya Institute of Technology Repository System.

Hiro K., Fukuoka H., Nakamura S., Wada T., & Fujiwara J. (2019). Negative pressures of detergents in the metal Berthelot tube. Matter: International Journal of Science and Technology, 5, 121-129. https://doi.org/10.20319/mijst.2019.53.121129

Hiro K., Ohde Y., & Tanzawa Y (2003). Stagnations of increasing trends in negative pressure with repeated cavitation in water / metal Berthelot tubes as a result of mechanical sealing. Journal of Physics D: Applied Physics, 36, 592-597. https://doi.org/10.1088/0022-3727/36/5/325

Hiro K., Wada T., & Kumagai S. (2014). Temperatures of maximum density in a pressure range from 15 MPa to -15 MPa generated for water in a metal Berthelot tube. Physics and Chemistry of Liquids, 52, 37-45. https://doi.org/10.1080/00319104.2013.793598

Hiro K., Imanishi M., Itsuki A., Nakamura S., Shimada H., & Fukuoka H. (2022). An application of a Berthelot method using a metal tube to inactivation of bacillus subtilis. International Journal of Biology and Biomedicine, 7, 30-33.

Imre A. R., (2002). Liquid-liquid phase equilibria in binary mixtures under negative pressure. In Imre A. R., Maris H. J., & Williams P. R. (Eds.), Liquids under nagetive pressure (pp. 81-94). Dordrecht: Kluwer. https://doi.org/10.1007/978-94-010-0498-5

Larios E., & Gruebele M. (2010). Protein stability at negative pressure. Methods, 52, 51-56. https://doi.org/10.1016/j.ymeth.2010.04.010

Ohde Y., Ikemizu M., Okamoto H., Yokoyama T., & Shibata S. (1989). Cavitation history effect of a water-metal Berthelot tube system interpreted by an elaborated gas-trapping crevice model. Journal of Physics. D: Applied Physics, 22, 1721-1727. https://doi.org/10.1088/0022-3727/22/11/023

Ohde Y., Komori K., Nakamura T., Tanzawa Y., Nishino Y., & Hiro. K. (2001). Effects of gas transports in metals on negative pressures in water in Mo/Cu Berthelot tubes. Journal of Physics D: Applied Physics, 34, 1717-1726. https://doi.org/10.1088/0022-3727/34/11/325

Ohde Y., Yasutoshi T., Motoshita K., & Hiro K. (2008). Thermobarometry for 4'-octylbiphenyl-4-carbonitrile in metal tube Berthelot method and polymorphism in crystalline phase of 4'-octylbiphenyl-4-carbonitrile found through cooling paths in negative-pressure range. Journal of Physics D: Applied. Physics, 47: 5591-5601. https://doi.org/10.1143/JJAP.47.5591

Okamoto H., & Ohde Y. (1986). Environmental stress failure: an irreversible thermodynamics approach. In Brostow W., & Corneliussen R. D., (Eds.), Failure of Plastics (pp.331-344). New York: Hanser.

Sarc A., Kosel J., Stopar D., Oder M., & Dular M. (2019). Removal of bacteria Legionella pneumophila, Escherichia coli, and Bacillus. Ultrasound Sonochemistry, 42: 228-236.

Smeller L. (2002). Pressure-temperature phase diagrams of biomolecules. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, 1595: 11-29. https://doi.org/10.1016/S0167-4838(01)00332-6

Speedy R. J. (1982). Stability limit conjecture. An interpretation of the properties of water. Journal of Physics Chemistry, 86: 982–991. https://doi.org/10.1021/j100395a030

Tamura T., Nakao M., Ogasawara T., & Takahira H. (2022). Numerical Simulation for cavitation inception for focused ultrasound using the Ghost fluid method coupled with bubble dynamics. Japanese Journal of Multiphase Flow, 36: 95-106. https://doi.org/10.3811/jjmf.2022.008

Trevena D.H. (1987). Cavitation and tension in liquids. Bristol: Adam Hilger. https://doi.org/10.1016/j.ultsonch.2017.11.004