Bacterial Sterilization Using Cavitating Jet*

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Abstract

In this paper, a new sterilization method using cavitating flow is presented. Water with bacteria was pressurized up to 105 MPa and flushed out through two very small nozzles 0.1-0.31 mm in diameter, where a cavitating jet was generated containing bubbles that collapsed downstream. First, the effects of jet velocity and cavitation number on the sterilization rate of *Escherichia coli* JCM1649T (*E. coli*) were examined. The sterilization rate increased with jet velocity. The rate was proportional to the 3rd power of the velocity. All the *E. coli* cells were killed by three successive treatments at \( V = 355.7 \) m/s and cavitation number \( \sigma = 0.154 \). The sterilization rate has a peak depending on cavitation number at the low-jet-velocity region of less than 300 m/s.

An experiment was also performed to compare two types of bacteria, *E. coli*, as typical Gram-negative bacteria and *Bacillus subtilis* JCM1465T (*B. subtilis*), as typical Gram-positive bacteria. Additional tests were performed using *Pseudomonas putida* JCM13063T, Gram-negative bacteria and *Bacillus halodurans* JCTM9153, Gram-positive bacteria. The sterilization rate of the Gram-positive bacteria was much lower than that of the Gram-negative bacteria under the same experimental conditions. Gram-positive bacteria have a thicker peptidoglycan layer than Gram-negative bacteria. This may be the reason why *B. subtilis* is more resistant to the mechanical stress caused by cavitating flow.

Key words: Sterilization, Bacteria, *Escherichia Coli*, *Bacillus Subtilis*, Cavitation, Water Jet, Peptidoglycan Layer

1. Introduction

Bacterial sterilization is one of the most important procedures in human life. Heating is the most common method of sterilization. However, it requires a considerable amount of energy, and sometimes the structure of the sterilized material is altered. Chemical sterilization is another popular sterilization method, but chemicals may have side effects on the human body. Recently, high-pressure processing (HPP) has become popular for food processing\(^{(1)-(3)}\). A high pressure of up to 1000 MPa can sterilize food without sacrificing freshness, flavor, and other properties.

In this paper, a completely different method of sterilization using a high-speed jet with cavitating is presented. Cavitation is a phase-change phenomenon from liquid to vapor, and is very similar to boiling\(^{(4)-(6)}\). Cavitation is induced by decreasing pressure in a system, whereas boiling is induced by increasing liquid temperature, which causes an increase in vapor pressure. Cavitation occurs in vortex rings generated in a shear layer of a jet flow\(^{(7)(8)}\). Usually, water contains many small air bubbles, which are sometimes called nuclei. When the nuclei enter the core region of vortex rings, they cavitate when the pressure is below the vapor pressure. This is called the inception of cavitation. When a cavitation bubble enters a
high-pressure region, it collapses intensely. The peak pressure caused by the collapse sometimes exceeds 1 GPa\(^9,10\). The collapse of cavitation bubbles causes the destruction of materials and the generation of noise and vibration. Therefore, it has been thought that cavitation is a harmful phenomenon, and should be avoided when designing and operating fluid machinery.

However, the generation of such a high pressure can be used for positive purposes, such as mixing, cleaning, and cutting. Vibratory cavitation has been used for the acceleration of chemical reactions, which is known as sonochemistry\(^11,12\). Recently, Soyama and coworkers\(^13,14\) proposed the use of a cavitating jet for improving the fatigue strength of metals. Cavitation is also widely used in biological and bioengineering fields\(^15\). The cavitating jet is promising for the remediation and disinfection of water, including the killing of bacteria. Hashiba et al.\(^16\) developed a prototype ultrasonic sterilizer, and succeeded in inactivating Cryptosporidium oosysts. Kalumuck et al.\(^17\) performed a sterilization experiment on Escherichia coli in a vessel using a cavitating jet. The cavitating jet method is much simpler than the ultrasonic method. The water is pressurized and ejected out through a nozzle or nozzles in the cavitating jet method. Kalumuck et al.’s result\(^17\), however, showed a low sterilization rate, although they did not describe the important experimental conditions, such as the jet velocity. It seems more than 100 treatments were required to realize perfect sterilization in their study. Recently, the authors have attempted to utilize cavitation for many aspects of environmental protection\(^18-21\).

In this paper, we present the result of sterilization experiments on two different types of bacterium, Gram-positive and Gram-negative. The experiment was performed in two stages. In the first stage, the effects of jet velocity and cavitation number on the sterilization of Escherichia coli JCM1649\(^T\) (E. coli), a typical Gram-negative bacterium, were examined. In the second stage, the effectiveness of sterilization on both Gram-positive and Gram-negative bacteria was examined and compared.

The advantage of using a high-speed water jet with cavitation for sterilization is that the process is very simple and reliable. We pressurize water containing bacteria, and force it out through small nozzles. No additional chemicals or treatment is needed. Therefore, the method is cheap, reliable, and safe. These are the greatest advantages of using cavitation.

2. Experimental Method

Most bacteria can be classified as Gram-positive or Gram-negative. The authors selected two typical types of bacterium, Bacillus subtilis JCM1465\(^T\) (B. subtilis) as a typical example of Gram-positive bacteria, and Escherichia coli JCM1649\(^T\) (E. coli) as a typical example of Gram-negative bacterium. E. coli are Gram-negative, motile, and facultative anaerobic bacteria. The shape is that of straight rods, measuring 2-4 x 0.4-0.7 micrometers, as shown in Fig. 1, whereas, B. subtilis are Gram-positive, motile, spore-forming and aerobic bacteria. The shape is that of straight rods, measuring 2-3 x 0.7-0.8 micrometers, as shown in Fig. 2.

Additional comparative experiments were performed using Pseudomonas putida JCM13063\(^T\) (P. putida), Gram-negative bacteria and Bacillus halodurans JCM9153 (B. halodurans), Gram-positive bacteria. P. putida \(^22,23\) is Gram-negative, motile, and aerobic bacteria. The shape is that of straight or slightly rods, measuring 1.5-5.0 x 0.7-0.8 micrometers. P. putida are organic-solvent-tolerant microorganisms, among them, the organic solvent tolerance of P. putida IH-2000\(^22\) and P. putida S-12\(^24\) is strong. These strains are tolerant to toluene.

B. halodurans C-125\(^25,26\) are Gram-positive, motile, and aerobic bacteria. The shape is that of straight rods, measuring 2-3 x 0.7-0.8 micrometers. B. halodurans C-125 are alkaliphilic bacteria, and the optimal pH for growth is 10.5.
Figure 3 shows the loop of the cavitating-jet experiment. A plunger pump can generate a very high pressure of up to 150 MPa. Water containing bacteria is ejected through two nozzles set in series, forming a cavitating jet. Figure 4 shows the details of the test section with two nozzles. The two nozzles’ diameter, together with the pump output, changes the flow velocity and pressure at the nozzle. Eight nozzles with different diameters, 0.10 mm, 0.13 mm, 0.16 mm, 0.20 mm, 0.23 mm, 0.25 mm, 0.28 mm, and 0.31 mm, were used. The diameter of the upstream nozzle is smaller than that of the downstream nozzle, so that the jet velocity at the upstream nozzle is greater than that of the downstream one.

We can control the velocity at the upstream nozzle and the pressure in the chamber between the two nozzles by changing the outlet pressure of the plunger pump and the diameter of the two nozzles. In the present experiment, the maximum velocity at the upstream nozzle was 384 m/s, and the cavitation number based on the upstream nozzle velocity and chamber pressure ranged between 0.037 and 0.487. The definition of cavitation number is given later in Eq. (1).

The test liquid (distilled water including bacteria) was placed in a vessel (see Fig. 3) then drawn into the plunger pump and pressurized. Then, the test liquid was flushed out through the two nozzles, where a cavitating jet was generated. Cavitation bubbles generated at the upstream nozzle collapsed more intensely than those generated at the downstream nozzle, because the pressure in the chamber was much higher than the outlet pressure (atmospheric pressure) of the downstream nozzle. Therefore, the governing parameters were upstream nozzle velocity and cavitation number.
The experiment was performed in two stages. First, the effects of jet velocity and cavitation number on the sterilization rate were examined using *E. coli*. Then, the sterilization rates were compared among two Gram-positive bacteria, *B. subtilis* and *B. halodurans*, and two Gram-negative bacteria, *E. coli* and *P. putida*.

The viscosity of the water containing bacteria was measured using a Cannon-Fenske viscometer. Figure 5 shows the result including that of pure water. The kinematic viscosity does not increase significantly with the existence of bacteria, so that we can use the fluid property of pure water as a first approximation.

The pressure in the chamber between the two nozzles was estimated using Bernoulli’s equation. Then, the mean velocity in the nozzle was estimated by assuming a flow coefficient of 0.90 on the basis of the estimated choke number (27).

3. Experimental Procedure

The authors used *E. coli* with tolerance to antibiotics to examine the effect of jet velocity and cavitation number on the sterilization rate. First, a Luria-Bertani (LB) liquid culture medium was sterilized in an autoclave, and Kanamycin (0.025 g/l) was added at a rate of 100 microliter/ml. Then, the LB liquid culture medium was hardened with agar in a disposable petri dish. *E. coli* with tolerance to antibiotics was incubated on the petri dish. This treatment is important to avoid possible contamination with other bacteria.

*E. coli* was incubated for 18-20 h at 37 degrees in an incubator shaker and its absorbance was examined using a spectrophotometer. The test liquid containing *E. coli* was used in the experiment after adjusting the value of infrared light absorbance (optical density 660 nm) to be between 1.5 and 1.7 in the sterilized LB medium. In this work, the cell density of *E. coli* was adjusted to be $1.4 \times 10^{11}$/ml on average.

The test liquid containing *E. coli* was placed in the supply vessel of the experimental loop, and flowed through the two nozzles in the test section (Figs. 3 and 4). The velocity in the nozzle and cavitation number were adjusted by selecting the diameter of the two nozzles and the stroke speed of the plunger. The same test liquid was tested 1 to 10 times successively, referred to as “one pass” to “ten passes”, respectively. After each pass, the test section and plunger pump were washed with a large amount of sterilized distilled water.

The effect of a cavitating jet on bacteria was measured by counting the colony-forming units (CFUs). The test liquid after each pass was diluted one-million-fold by the liquid medium. Then, 50 microliters of the diluted test liquid was spread on a solid medium and incubated for 18-20 h at 37 degrees in an incubator, resulting in the formation of colonies. Figure 6 shows an example of CFUs on a petri dish, which were counted by the naked eye. The numbers “0” to “5” on the photograph in Fig. 6 represent the number of passes. It is clearly seen that the number of colonies is decreased monotonically with each successive treatment.

When the dilution was insufficient, there were too many colonies to perform an
accurate count. One-million-fold dilution was adequate, as seen in Fig. 6. Two independent samples of colonies on the solid medium were made, so that the possible experimental errors could be avoided. The reliability of the result was also examined by repeating the experiment 10 times independently under the same conditions, \( V = 242.3 \text{ m/s} \) and cavitation number = 0.105. The mean sterilization rate after 3 passes was 0.962, and the standard deviation was 3.6%. The reliability of the experiment was satisfactory.

The present experiment was performed in the laboratory of level P2 on the Kawagoe campus, Toyo University. All the treatments were carried out on a clean bench to avoid possible contamination by other bacteria. Experimental materials such as petri dishes, test liquid, distilled water after washing, and paper towels were sterilized in an autoclave to avoid possible biological hazards. The experimental apparatus (Fig. 3) was too large to sterilize in the autoclave, so the flow chamber and inside pipes were sterilized with 30% \( \text{H}_2\text{O}_2 \) in water. The duration of sterilization was the same as that of one pass; a few minutes. The exterior was sterilized by spraying ethyl alcohol. After sterilization by \( \text{H}_2\text{O}_2 \) and ethyl alcohol, we flowed distilled water through the test section and confirmed that there were no live bacteria in the distilled water by incubating a small amount of the water.

![Fig. 7 Cavitating jet in enlarged model (Re=2.89 x 10^4, \( \sigma = 0.43 \))](image)

### 4. Experimental Results

As mentioned above, *E. coli* with tolerance to antibiotics was used as the test organism. We changed the jet velocity in the upstream nozzle from 176 m/s to 384 m/s, and the cavitation number from 0.037 to 0.487. Cavitation number (\( \sigma \)) was defined as

\[
\sigma = \frac{2(p - p_v)}{\rho V^2},
\]

where \( p \) and \( p_v \) are the pressure of the chamber between the two nozzles and the vapor pressure, respectively. \( \rho \) and \( V \) are the water density and water velocity in the upstream nozzle, respectively.

The cavitation inception is governed by the cavitation number, Reynolds number, the amount of nuclei in the flow, and other parameters\(^\text{(4,5)}\). The inception cavitation number of a submerged water jet of normal size is about 0.6\(^\text{(28)}\). However, the cavitation inception of a water jet from a small nozzle less than 1 mm in diameter decreases substantially\(^\text{(29)}\). This was because the flow becomes laminar and the number of nuclei decreases, because the number of nuclei is proportional to the flow rate.

In the present experiment, the flow velocity is much higher than in the case of reference 29. The Reynolds number, based on the nozzle diameter and the mean velocity in the nozzle, was more than \( 2 \times 10^4 \). Therefore, cavitation must occur earlier than in the case of reference 29. The authors tried to visualize the jet flow from the nozzle. However, it was not possible because of the high pressure and narrow space. Instead, a test section with a 10-fold-enlarged nozzle was fabricated and we observed the cavitating jet with the same Reynolds and cavitation numbers as those of the prototype. Figure 7 shows the cavitating jet from the enlarged nozzle with \( \text{Re} = 2.89 \times 10^4 \) and \( \sigma = 0.43 \). Note that almost all the experiments on bacteria sterilization were performed at a cavitation number lower than \( \sigma = \ldots \)
0.43. (See Fig. 16.) This suggests that cavitation should occur more in the sterilization experiment. However, the flow rate in the nozzle of the sterilization experiment is less than that of the enlarged nozzle, because the diameter of the nozzle is one tenth that of the enlarged nozzle shown in Fig. 7. This reduces the inception cavitation number, as mentioned by Oba et al. (29).

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**Fig. 8.** Sterilization rate vs number of passes for *E. coli*

**Fig. 9.** Survival rate vs number of passes for *E. coli*

**Fig. 10.** Sterilization rate against velocity and cavitation number for *E. coli*
Figure 8 shows the effect of the cavitating jet on the sterilization of *E. coli*. The sterilization rate increases with jet velocity regardless of the cavitation number. The effect of the cavitation number is not as clear as that of jet velocity. This will be discussed later.

The effect of the cavitating jet becomes clearer when the data are shown on a semilog scale of the survival rate, as shown in Fig. 9. The survival rate is defined as (survival rate = 1-sterilization rate). The data agree well with the log curve, which is shown as a straight line on the semilog diagram. The slope of the line shows the sterilization rate. The most effective condition is $V = 355.7$ m/s and $\sigma = 0.154$. All the *E. coli* cells appear to have been killed after three successive treatments. The two independent colony counts were in good agreement. It was also found that the sterilization rate is lower when the initial density of the *E. coli* is higher.

Fig. 11 Sterilization rate of various bacteria at $V=271.0$ m/s, $\sigma=0.104$, and $P=50$ MPa

Fig. 12 Sterilization rate of various bacteria at $V=353.7$ m/s, $\sigma=0.037$, and $P=80$ MPa

Fig. 13 Survival rate of various bacteria at $V=271.0$ m/s, $\sigma=0.104$, and $P=50$ MPa

Fig. 14 Survival rate of various bacteria at $V=353.7$ m/s, $\sigma=0.037$, and $P=80$ MPa
Figure 10 shows the sterilization rate of *E. coli* against the velocity and cavitation number at the upstream nozzle. When the velocity is lower than about 300 m/s, the sterilization rate has a peak at about $\sigma = 0.1$. On the other hand, when the velocity is higher than about 300 m/s, there seems to be no peak against cavitation number. This will be discussed in the next section.

Figures 11 and 12 show the result of the experiment comparing Gram-positive bacteria (*B. subtilis* and *B. halodurans*) and Gram-negative bacteria (*E. coli* and *P. putida*) under two conditions. It is clearly seen that the Gram-positive bacteria are stronger than the Gram-negative bacteria under both conditions. This is also evident in the log survival rate plots shown in Figs. 13 and 14.

5. Discussion

5-1 Effect of nozzle velocity and cavitation number

The destruction of bacteria is caused by mechanical stress in the present experiment. There seem to be two major mechanisms. The first mechanism is shearing stress caused by shear flow in the nozzle. The second mechanism is the shock wave generated by the cavitation bubble collapse. The high static pressure has the effect of bacterial sterilization, as mentioned in the introduction. However, the maximum static pressure is one order of magnitude less than that of high-pressure processing (HPP)(1-3). Therefore, the high pressure itself cannot be the cause.

We define the reduction rate (RR) of bacteria as
Then, all the data for *E. coli* shown in Fig. 10 are plotted as the log of RR against the log of nozzle velocity regardless of cavitation number. Figure 15 shows this plot. As expected, RR depends strongly on nozzle velocity. The slope is about 3 – 5; that is, RR is proportional to the 3rd-5th power of velocity. The erosion on a solid surface caused by cavitation bubble collapse is proportional to the 6th-8th power of velocity\(^{(4,5,30)}\). The present result does not indicate a very strong dependence on velocity.

Here, we assume that RR shown in Fig. 15 results from two mechanisms, the shear stress caused by shear flow and the shock wave caused by cavitation bubble collapse.

Assuming the effect of velocity on RR as
\[
\log (RR) = 3 \log (V) - 2.3, \tag{3}
\]
we subtract the value of Eq. (3) from the experimental value shown in Fig. 15, and plot the remainder against cavitation number, as shown in Fig. 16. There is a clear peak at a cavitation number of about 0.1. This confirms the discussion based on Fig. 10. The maximum value of \(\log RR\) is about 0.7, as seen in the figure, which roughly corresponds to \(\log (V) = 2.53\) in Eq. (3) and to about \(V = 340\) m/s in Fig. 15. This simple analysis also reconfirms that the effect of shear flow becomes dominant when the velocity in the nozzle becomes more than about 300 m/s.

5-2 Effect of shearing stress

Bulut et al.\(^{(31)}\) examined the effect of shearing stress on *Microbacterium lacticum* using a twin-screw extruder. Log RR increased roughly with the square of wall shear stress. The log RR values were about 1 and 4 at shearing stresses of 200 kPa, and 400 kPa, respectively.

The maximum shearing stress should appear at the inner wall of the nozzle in the present experiment. For example, the average velocity and cavitation number are 353.7 m/s and 0.037, respectively, at the upstream nozzle of 0.10 mm diameter and the plunger pressure of 80 MPa (the same conditions as shown in Fig. 12). The Reynolds number based on the diameter of the nozzle is \(3.54 \times 10^4\). Therefore, the inner flow of the nozzle should be turbulent. Applying the equation of shearing stress in a turbulent inner flow, the shearing stress at the wall is estimated as 361 kPa. The measured value of \(\log RR\) is 1.21 under this condition.

According to Bulut et al.’s result, the value of \(\log RR\) is about 3 at the shearing stress of
361 kPa. Although we cannot directly compare the present result with Bulut et al.’s empirical result, the present value of log RR is lower than Bulut et al.’s result. If Eq. (3) is valid, log RR is 0.74, which is one-fourth of Bulut et al’s result. This might be because the length of the nozzle is short (about 1 mm), so that the time of shearing stress is very short, on the order of 0.1 ms.

5-3 Difference between Gram-positive and Gram-negative bacteria

_E. coli_ are Gram-negative bacteria and the cell wall is not very resistant to mechanical stress. In contrast, Gram-positive bacteria have a more resistant cell wall than Gram-negative bacteria. Figure 17 shows the cell wall structures of both bacteria. Gram-positive bacteria have a thick peptidoglycan layer, which is resistant to mechanical attacks such as shear stress or shock waves. In contrast, Gram-negative bacteria have a thinner peptidoglycan layer, which is less resistant to mechanical attack.

It will be important and interesting to examine the effect of cell wall structure on sterilization using a cavitating jet.

![Fig. 18. Pressure change in test section](image)

5-4 On the instrument and future work

As shown in Fig. 4, the bacteria pass through two nozzles in the test section, where a cavitating jet is generated. The upstream cavitating jet collapses in the test section chamber between the two nozzles. The downstream nozzle maintains the high static pressure in the chamber. The cavitating jet collapses more intensely at a higher static pressure. Therefore, the governing parameters of sterilization are jet velocity and cavitation number at the upstream nozzle. In fact, these parameters correlate well with sterilization rate, as shown in Figs.15 and 16. A poor correlation, on the other hand, was found between sterilization rate and the downstream jet velocity and cavitation number.

The true mechanism of sterilization is not clear at present. As mentioned above, the upstream cavitating jet seems to be the principal mechanism. The shearing stress in the nozzle and the high pressure caused by the cavitation bubble collapse seem to be the most probable mechanisms. However, the large pressure drop through the nozzles and the shearing force in the jet flow are other possible causes of sterilization. Intensive studies should be conducted to determine the true mechanism of the sterilization of bacteria.

The present experimental loop (see Fig. 3) is pressurized using a single-stroke plunger pump. The plunger moves reciprocally in the cylinder, and stops at both ends. Therefore, pressure changes with time, as shown in Fig. 18. This means that pressure, as well as flow velocity, becomes very low at both ends, which markedly decreases the sterilization effect.

This shortcoming can be rectified easily by replacing the single-stroke plunger with a multiplunger pump (for example, three plungers), so that jet velocity can remain constant with time.

The present paper focuses on the effect of the flow conditions on the sterilization of bacteria using a cavitating jet. In principle, the present method can be used to sterilize fluids other than water. Thus, this method has a wide application potential.

A method for removing lipopolysaccharide (LPS) from the sterilized fluid should be developed for the practical use of the present method. LPS is a lipid-polysaccharide
complex, and inhabits the cell outer membrane of rod-shaped Gram-negative bacteria. Large amounts of LPS inoculated into the human body cause a variety of heart problems, such as a hypotensive effect and circulatory system disease by the decrease in the heart rate.

6. Conclusions

The effect of a cavitating jet on bacterial sterilization was examined experimentally. A flow system with two successive nozzles is very effective for bacterial sterilization. The upstream nozzle generates a cavitating jet downstream, which collapses in the chamber between the two nozzles.

The following conclusions were obtained.

(1) Sterilization rate increases with cavitating jet velocity at the upstream nozzle. This might be due to the high shearing stress in the nozzle. The high-pressure wave caused by cavitation bubble collapse seems to be another mechanism of sterilization.

(2) The sterilization rate was proportional to the 3rd power of the velocity. All the \emph{E. coli} cells were killed after three successive treatments at \(V=355.7\) m/s and cavitation number \(\sigma=0.154\). The log reduction rate of \emph{E. coli} was 1.21 in this case.

(3) The sterilization rate has a peak with respect to cavitation number at the low-jet-velocity region of less than 300 m/s.

(4) The sterilization rate of \emph{B. subtilis}, typical gram-positive bacteria, is much lower than that of \emph{E. coli}, typical Gram-negative bacteria. This may be because \emph{B. subtilis} has a thicker peptidoglycan layer than \emph{E. coli}, which is much more resistant to mechanical force.

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