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Shock Waves

Repeated application of shock waves as a possible method for food preservation

A.M. Loske*, F.E. Prieto*, M.L. Zavala, A.D. Santana, E. Armenta

Instituto de Física, UNAM, A.P. 20-364, 01000 México D.F., Mexico. E-mail: loske@fenix.ifisicacu.unam.mx

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Abstract. In order to study the possibility of using underwater shock waves to cause death in non desired microorganisms found in certain foods, *Escherichia coli* in suspension was exposed to hundreds of shock waves on an experimental electrohydraulic shock wave generator. Using a parabolic reflector it was possible to produce a plane shock front and expose many test tubes to the action of the shock waves at the same time and under the same conditions. The amount of surviving bacteria was determined by plate counting for different numbers of applied shock waves. Pressure measurements using needle hydrophones are also reported. Experimental results indicate that electrohydraulically generated shock waves are capable of producing a significant reduction in an *E. coli* population. An increase in the applied shock wave number produced a nearly exponential reduction in the *E. coli* population.

Key words: Food preservation, Effect of shock waves, *Escherichia coli*

1 Introduction

Since its introduction in 1980, extracorporeal shock wave lithotripsy (ESWL) has become the standard treatment for the majority of patients with renal and ureteral calculi (Chaussy et al. 1980; Loske and Prieto 1998) and an alternative in the treatment of gallbladder stones (Nahrwold 1993), pancreatic concrements (van der Hul et al. 1993), and stones of the salivary gland (Hessling et al. 1993). New clinical applications of shock waves are the treatment of non-union fractures (Haupt et al. 1992), as well as the management of pseudarthrosis (Schleberger and Senge 1992), tendinopathy and other orthopedic diseases (Haupt 1997). The treatment of tumors with shock waves is another experimental approach (Oosterhof et al. 1991). It has been shown that colony growth of tumor cells decreases as shock wave number increases (Berens et al. 1989). Unfortunately the tumor growth suppression observed *in vivo* is temporary.

Because of the successful applications of shock waves to medicine (Loske and Prieto 1995), low intensity underwater shock waves and the behavior of materials under the influence of low pressure shock waves received increased attention in the last fifteen years. For the same reason, the effects of shock waves on living cells have also been the subject of many investigations (Delius 1994; Loske and Prieto 1995).

The destructive effects of ultrasonic waves on bacterial cells, known for many years (Davies 1959), and the damages on living cells observed during ESWL (Delius et al. 1988), lead to the idea of using underwater shock waves as a possible method for food preservation.

In the food industry, heat treatments are commonly used to inactivate pathogenic microorganisms. Nevertheless, because heat may affect the organoleptic and nutritional characteristics of food, there is great interest in non thermal processes like ionizing irradiation (i.e. γ, β, and X rays), addition of preservatives, cold storage, pulsed electric fields, oscillating magnetic fields, high hydrostatic pressure and intense light pulses (Downing 1989; Mertens 1994; Pothakamury et al. 1993; Barbosa-Cánovas et al. 1994; Qin et al. 1995; Russell 1982). Some of these techniques are still being explored as possible alternatives.

It is the purpose of our investigation to evaluate the possibility of using underwater shock waves in order to cause death in non desired microorganisms found in certain foods, preventing them from carrying out the biological processes necessary for their existence and proliferation. This article reports our first results of the effects on *Escherichia coli* (*E. coli*) ATCC (American Type Culture Collections) 10536 under the action of weak underwater shock waves, generated with an experimental electrohydraulic shock wave generator.

Although in the device here described an electric discharge in water is used to produce the shock waves, this does not mean that the method is similar to the use of high voltage pulsed electric fields to preserve foods. In our case, microorganisms are not exposed to an electric field.

* *Present address:* Laboratorio de Choques Débiles, Física Aplicada y Tecnología Avanzada, Universidad Nacional Autónoma de México, A.P. 1-1010, Santiago de Querétaro, Querétaro 76000, México

As often in this kind of experiments, *E. coli* ATCC 10536 was chosen as the first microorganism to study the effect of electrohydraulically generated shock waves, because it is a well known and easy to handle bacteria. Additionally, the comparison with the results obtained by Kerfoot et al. (1992) and by Teshima et al. (1995) promised to be interesting.

2 Material and methods

2.1 The experimental shock wave generator

A new experimental underwater shock wave generator, named MEXILIT II was designed and constructed. The MEXILIT II, similar to its former version (Prieto et al. 1991), consists of a pulsed power circuit, operating between 10 and 1000 Joules, providing multiple pulses to a spark gap immersed in water. The spark gap electrode assembly is at the focal point of a parabolic stainless steel reflector, with a focal distance of 20.0 mm, a *latus rectum* of 80.0 mm, a maximum internal diameter of 172.0 mm and a depth of 92.5 mm (see Fig. 1), mounted on the bottom of a 1200×800×600 mm Fiberglass water tank (see Fig. 2). Application of high voltage (up to 30 kV) across a pair of electrodes induces a spark, creating the sudden ionization of the water. The fast expansion of the gas bubble generates a shock wave, propagating into the surrounding water and reflecting off the reflector, creating a plane shock front. The MEXILIT II, shown in Fig. 2, has the Fiberglass water tank mounted on an iron frame. A three dimensional computer controlled position system is placed on top of the device, in order to fasten and move any probe, pressure transducer, test tube or sample to any desirable position within the tank. Basically the electric circuit consists of a computer controlled capacitor charging system and a discharge device. In this work, capacitance and voltage were set to 80 nF and 20±0.1 kV respectively. The spark gap was set to one millimeter. Electrodes, with the shape of a truncated cone (Loske and Prieto 1993), were allowed to burn in for 400 discharges at 18±0.1 kV. Tap water having a conductivity of 960±5 microsiemens/cm and a temperature of 27±0.1°C was used. Basically, the MEXILIT II is similar to electrohydraulic shock wave lithotripters used in ESWL, except for having a parabolic reflector instead of the ellipsoidal reflector used for clinical applications.

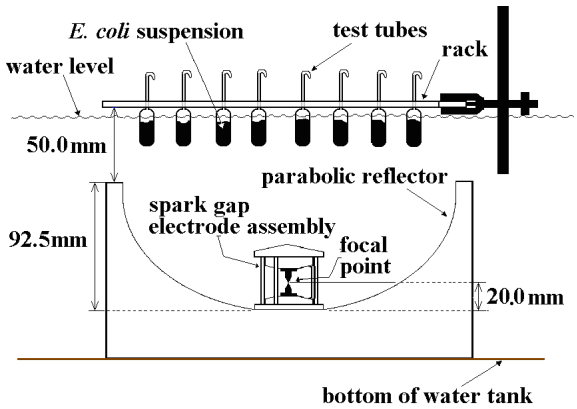

Fig. 1. Sketch of the parabolic stainless steel reflector with the spark gap electrode assembly at its focal point and the rack holding the test tubes

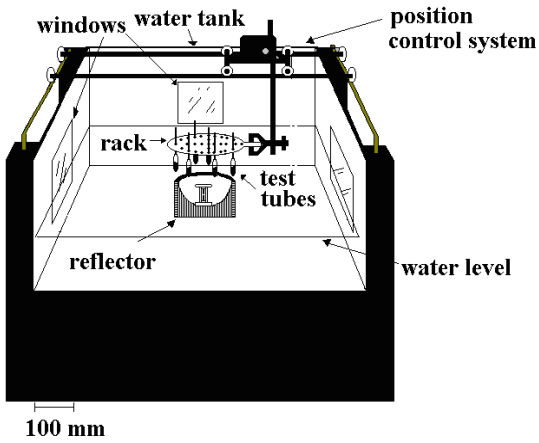

Fig. 2. Sketch of the MEXILIT II electrohydraulic shock wave generator. For clarity the front side of the water tank was not drawn

2.2 Sample preparation

A 24 hr at 37°C culture of *E. coli* ATCC 10536 in nutritive broad (Merck V552243-448) was used. After cultivation, the cells were collected by centrifugation and resuspended in a 0.9% NaCl solution. After three washes using the same procedure, a suspension containing 10^5–10^7 CFU/mL was prepared.

The concentration of bacteria before cultivation and after the washes was registered and adjusted with a photometer (Lakeside Mannheim Boehringer model 4010) at 623 nm, using media and 0.9% NaCl solution as a blank. The first was only to confirm the culture growth, and the second to dilute the sample with saline solution until the reading corresponding to the desired bacteria concentration was obtained.

A total of 56 disposable test tubes (Elkay Products Inc., model 127-P507-STR) were filled and heat sealed. Half of the pipettes were placed inside the water tank of the MEXILIT II. The other 28 test tubes remained as control samples in a separate water bath at the same temperature, for the same time as each of the "treated" test tubes. As can be seen in Figs. 1 and 2, the pipettes were placed on a specially designed plane circular Lucite rack, capable of holding 28 tubes at the same time. The rack was fastened with an ordinary laboratory clamp to the position control system of the MEXILIT II at an arbitrary distance of 122.5±0.5 mm from the focus of the reflector, which corresponds to a 50 mm separation between the upper border of the reflector and the Lucite rack (Fig. 1). All samples were positioned so that their center was 107.5±0.5 mm from a

horizontal line (*latus rectum*) going trough the focus of the reflector. Shock waves were generated at a frequency of 0.4 Hz. The water level was 45 mm over the border of the reflector.

2.3 Experimental procedure

The experiment was repeated five times. Each time a total of 2000 shock waves were applied to the rack holding the test tubes. After every 500 shock waves, four test tubes were randomly taken out of the shock wave generator, mixed in a flask and identified. The same procedure was applied simultaneously to the 28 control tubes, not exposed to the shock waves.

The following biochemical analysis were performed in order to detect possible changes in the *E. coli* metabolism: Kligler, H_2S, citrate, mobility, indole and urea.

Samples were serial-diluted (1:10) and the amount of surviving bacteria determined by plate counting (agar plate count: Merck V877063708). The number of colony-forming units per milliliter (CFU/mL) obtained to fill the pipettes before treatment, was used as a sample for zero discharges.

2.4 Pressure measurements

The pressure applied was recorded using a needle hydrophone (Imotec, GmbH, Würselen, Germany) with a 20 ns rise time. Signals coming from the gauge were sent to the input channel of a *Tektronix 2430A* digital oscilloscope (Tektronix, Inc., Beaverton, Oregon), placing the hydrophone at the axis of symmetry of the parabolic reflector, at 107±0.5 mm from the focus, and also at ten other positions, moving the transducer horizontally away from the axis in 9 mm steps. Two hundred measurements were recorded at each position. A new set of electrodes was used for each position and allowed to burn in for 400 discharges at 18 kV. In order to measure the pressure drop due to the test tubes, the *Imotec* pressure gauge was placed inside the water tank at 100±0.5 mm from the spark gap. After burning in the electrodes, 50 pressure profiles were recorded without covering the gauge. After that, the gauge was immersed in an inactivated *E. coli* suspension inside a test tube and placed at the same position in order to take another set of 50 measurements. All measurements were done at the voltage, capacitance, water temperature and conductivity already mentioned in 2.1, using the cursors of the digital oscilloscope, and fed into a personal computer for carrying out the statistical analysis.

In order to save time while measuring with the cursors of the oscilloscope, all rise times were defined as the time required for the wave to rise from the baseline to the maximum amplitude and not in the conventional way, as the time required to rise from 10% to 90% of the maximum amplitude. For the same practical reasons, the widths were measured at the baseline and defined as the time from the instant where the pulse rises, to the instant where it crosses the baseline again. This should not be confused with some reported data, using ellipsoidal reflectors, where the width is defined as the time over which the pressure is greater than one half of the peak compressional pressure pulse. The implications of using these definitions of the pulse rise time and widths are explained in the Discussion section.

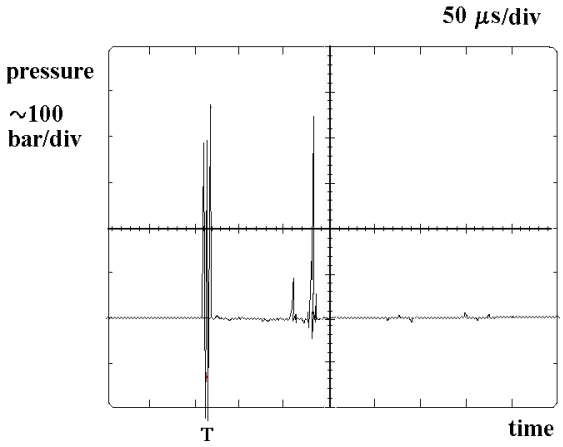

Fig. 3. Pressure record obtained using a needle hydrophone at about 107 mm from the *latus rectum* of the parabolic reflector shown in Fig. 1 and at about 18 mm from its axis of symmetry

3 Results

Figure 3 shows a typical pressure record obtained at 107± 0.5 mm from the focus of the reflector and at 18±0.5 mm from its axis of symmetry. The signal was obtained using a 50 μs/div time base. Each vertical division corresponds to about 10 MPa. The electromagnetic signal of the high voltage discharge can be seen at the beginning of the trace at the instant (T) when the oscilloscope was triggered. The direct shock wave arrives after about 84 μs and is followed approximately 22 μs later by the reflected pressure wave. All pressure variations, recorded at the other positions, showed a similar behavior.

The average peak positive pressure of the reflected pulse, corresponding to the first ten transducer positions was 44±7 MPa, having a width and rise time of 4±0.5 μs and 2.8±0.1 μs, respectively, followed by a negative pressure pulse of 6±3 MPa. A statistical analysis revealed no significant difference between pressure measurements at the different positions, except for the last two at 81 and 90 mm from the axis of symmetry of the reflector, were the positive pressure dropped about 25 and 40%, respectively. This is probably due to diffraction of the pressure wave at the borders of the reflector. Because of this, the mentioned average pressure values refer only to the pressure at the axis of symmetry and the first nine consecutive positions. No test tubes were located at more than 65 mm from this axis.

The measured pressure drop due to test tubes filled with cell suspension was about 20%.

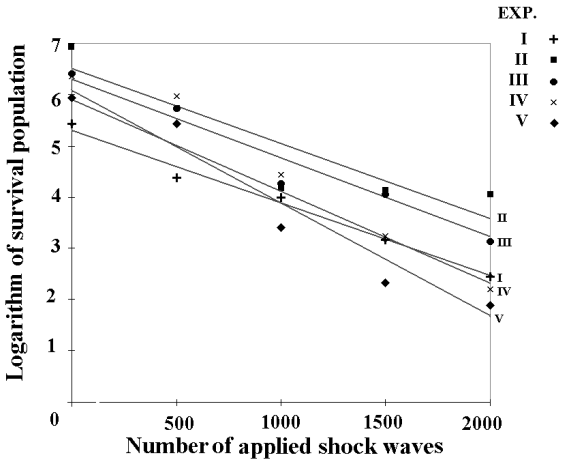

Fig. 4. Graphs of the logarithm of survival *E. coli* population vs. the number of applied shock waves for five experiments. Least squares linear fits to the experimental results are shown

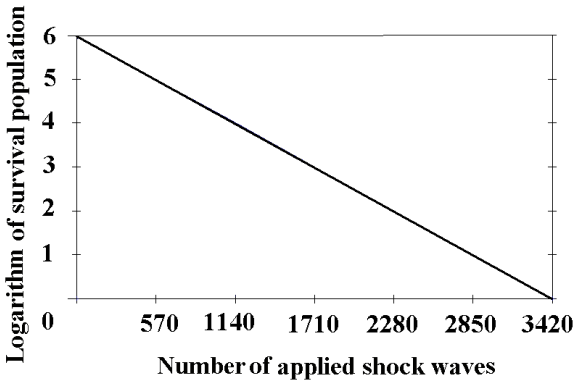

Fig. 5. Expected behavior of *E.coli* growth after shock wave exposure

Biochemical analysis did not reveal any change in the metabolism of the *E. coli* microorganisms.

Results indicate a nearly logarithmic reduction in the microorganism population after shock wave exposure. In order to determine the mortality index of the exposed *E. coli* ATCC 10536 bacteria, an initial count between 10^5 and 10^7 CFU/mL was used. This value is comparable to the concentration reported by the Association of Official Analytical Chemists for some contaminated food products (Analytical Chemists, vol. I, 15th edition, Washington DC, Association of Official Analytical Chemists, Inc. (1990) pp 435–436, 803–805).

Figure 4 are the graphs of the logarithm of the survival population vs. the number of applied shock waves for the five experiments, showing a similar slope K. Generally, K, referred to as velocity constant or mortality index (Block 1994) is obtained using the formula

$$N = N_0 \mathrm{e}^{-Kt} ,$$

where t stands for time in minutes, N_0 for initial number of microorganisms and N for number of microorganisms which survived after t minutes. In this study, results are given in dose or "applied shock waves", instead of time. This is due to the fact that the shock wave generation frequency is a parameter which can be set and modified depending on the selected voltage and capacitance of the shock wave generator. In this experiments the mean value of K was 0.0018, with a coefficient of variation (standard deviation divided by the average) of only 0.13.

The average dose $D = t/(\log N_0 - \log N)$, needed to reduce the initial amount of microorganisms 90% was about 569 shock waves, having a coefficient of variation of 0.14.

Figure 5 is a graph of the logarithm of the survival population vs. the applied shock wave number, showing the expected behavior continued to 6 D. The straight line has a slope $K = 0.0018$. This means that the reduction seems to follow an exponential behavior.

Since in this case a frequency of 0.4 Hz was used, it would take about 24 minutes to generate 569 shock waves. This means that it would be necessary to apply electrohydraulic generated shock waves (at the already mentioned voltage, capacitance and frequency) for about 24 minutes to reduce the *E.coli* population from 10^6 to 10^5 CFU/mL. In order to inactivate the initial population, 6 D or about 143 minutes are needed (Block 1994).

4 Discussion

The cell container and environment around and within the cell tube are important because they will directly influence on the transmission of the shock wave to the cells. Polypropylene was chosen for the test tubes because its acoustic impedance approximates that of water. Nevertheless, pressure measurements revealed that the shock wave lost about 20% of its value when passing through the test tube. Pipettes with thinner walls or made out of a different material, could reduce this pressure attenuation.

Spark gaps in water generate broad band pressure pulses with very short rise times and high pressures which depend on several parameters, some of which can be controlled and some can not. The reported variations in pressure measurements are typical of electrohydraulic shock wave generators (Coleman and Saunders 1989; Prieto et al. 1994). These variations did not affect our results because microorganisms were exposed to hundreds of shock waves.

The electrode tips of the shock wave generator wear off due to the high temperatures and forces acting on them during each electric discharge. As a result of this erosion, the electrodes have a limited lifetime. In order to reduce time between voltage application and spark gap generation, the electrode gap should not exceed 3 mm (Loske and Prieto 1993). Furthermore, as the electrode gap becomes larger, the pressure of the shock wave increases. Additionally, in general the electric spark gap does not link the two electrodes by the shortest path. Therefore, the electric discharge is rarely located at the focus and leads to dispersed pressure peaks around the second focus of ESWL

lithotripters. This can be improved by axial positioning of the electrodes in the reflector, as in the MEXILIT II. Considering a 400 discharge burn in at 18 kV, the practical lifetime of the electrodes was estimated to be about 2400 shock waves at 20 kV, using an 80 nF capacitance. Pressure measurements have shown that between 400 and 2400 discharges, the pressure profile is fairly constant. Beyond 2400 shock waves, the pressure amplitude variation, as well as the number of misfires, increase significantly. Due to this, the maximum number of applied shock waves was 2000. The extrapolation of our experimental data (Fig. 5) revealed that a total of about 3420 shock waves are needed to completely inactivate the bacteria. In order to replace the worn-off electrode with a new one, it is necessary to empty the water tank of the MEXILIT II. It takes about 30 minutes to empty the tub, replace the electrode, fill the tank again, adjust the water temperature and conductivity to the desired values and burn in the new electrode. During this process, the pipettes would have to be taken out of the shock wave generator and placed in a separate water bath having the same temperature. After that, the experiment could be continued until 3420 shock waves have been administered. Since a 30 minute waiting time would significantly alter the results, the electrode was not changed and the experiment was stopped after 2000 shock waves. In the future, this shortcoming could be solved using a different type of electrodes or using an *ellipsoidal* reflector in order to increase the pressure and reduce the number of shock waves needed to perform an experiment with a D6. This could reveal the existence of bacteria that were originally resistant to shock waves or became so during shock wave treatment. As already explained, the disadvantage of using an ellipsoidal, instead of a parabolic reflector, is that only *one* pipette should be placed at the second focus and exposed to the shock waves at a time. This significantly increases the experimentation time. If shock wave application reveals to be a convenient method to be used in the food or pharmaceutical industry, other shock wave generation mechanisms will have to be developed.

Shock waves from electrohydraulic generators are considered weak. Nonlinear effects appear only in the proximity of regions where the energy is concentrated. This is the case in extracorporeal lithotripters, using ellipsoidal reflectors, but not in this study, where a parabolic reflector was used.

It is important to point out that the radiant output of the underwater spark has a continuum in the ultraviolet (UV), having a peak at approximately 55 to 150 nm. This ultraviolet radiation could contribute to microorganism death. Nevertheless, the intensity of this radiation is reduced significantly during its path through the water and the test tube. The influence of this UV radiation on the reduction of microorganism population is currently been studied. Experiments on human tumor cells, exposed to electrohydraulically generated shock waves using opaque polypropylene pipettes, have shown no evidence of cell death due to UV light (Berens et al. 1989). Obviously this result could be different when using *E. coli*. The fact that Ohshima et al. (1991) found that the intact cells of *E. coli* JM 109/pKPDH2 are difficult to be destroyed by shock waves using a shock tube which does not generate UV light, indicates a possible influence of the spark-generated electromagnetic radiation.

Even if it is known that *E. coli* can grow at static pressures up to 55 MPa, the response to dynamic pressures is expected to be different, since in this case there is not an even distribution of pressure in the cell suspension. Furthermore, static pressures do not produce cavitation in the suspension. Cavitation is generated whenever there is a rapid transformation of positive pressure into tensile stress. In the MEXILIT II, the pressure wave initially produces a high positive pressure, which is rapidly transformed into tensile stress within microseconds, resulting in the formation of vapor-filled cavities. These cavities implode, creating very high energy densities.

In general, microorganisms can be killed by static pressure of about 100 MPa, but the complete sterilization is often difficult because of so called "persisters". These are many reasons why simple compression and decompression does not harm microorganisms in the same way as the repeated administration of a very short high pressure pulse followed by a negative pulse. Cavitation depends on the pressure of the medium, the presence of microbubbles in the sample and the existence of a liquid-air interface. The mechanism by which cavitation may cause biological damage are high localized temperature and pressure gradients.

The bactericidal effect of ultrasound has been attributed to cavitation (García et al. 1989). It is interesting to point out that the increase in human renal cell carcinoma xenografts loss in tubes containing air was reported to be 40% higher as compared to sample tubes without air (Steinbach et al. 1992). This might be explained by an increased occurrence of transient cavitation, caused by reflection of the pressure wave at the liquid-air interface. The interface results in perturbation in the shock front with resultant surface shear and cavitation within the suspension. It is for these reasons that the microorganism death is expected to reduce in the absence of a liquid-air interface. In our case, the test tubes were only filled up to about 75%.

As far as we know, Kerfoot et al. (1992) did the first experiments designed to isolate the effects of shock waves on bacterial cells (*Pseudomonas aeruginosa, Streptococcus faecalis, Staphylococcus aureus and Escherichia coli*) and determine whether bactericidal activity exists. In this study, the suspension received 200 shock waves at 20 kV and a rate of 100 shocks per min on a HM3 Dornier electrohydraulic lithotripter (Dornier Medizintechnik GmbH, Germering, Germany). The experiment was repeated delivering 4000 shock waves at the same energy and rate. Aliquots of bacterial suspensions of each of the four bacterial strains were also exposed to 4000 shock waves generated by a Wolf Piezolith 2200 piezoelectric lithotripter, which does not generate UV radiation, at energy level 4 and a rate of 120 shock waves per min. Contrary to our results, the authors concluded that shock waves do not possess significant bactericidal activity. It is important to notice that, even if the MEXILIT II shock wave genera-

tor is capable of reproducing the pressure field generated by a HM3 lithotripter, in our study, at 20 kV the cells received less energy due to the fact that a *parabolic* reflector was used instead of the conventional ellipsoidal reflector of the HM3. Since Kerfoot et al. filled each test tube completely in order to exclude air bubbles, we conclude that the bactericidal effect observed in our study is due to shock wave reflection and cavitation at the air-fluid interface. Cavitation may also explain reports of decreases in both persistent urinary tract infection and bacteriuria after ESWL of infection stones (Michaels et al. 1988; Pode et al. 1988). Experiments using aerated fluids in order to enhance cavitation in the cell suspensions, could show a strong bactericidal effect of shock waves.

Since the resistance of some microorganisms to heat is reduced by previous treatment with ultrasound (Burgos et al. 1972) a combination of shock wave and heat treatment should also be investigated in the future.

As already mentioned, Ohshima et al. (1991) found that the intact cells of *E. coli* JM 109/pKPDH2 are difficult to be destroyed by shock waves. They confirmed that these cells of *E. coli* were killed predominantly when small bubbles were introduced into the cell solution. Nevertheless it has to be noticed that they used a shock tube, which generated a positive pressure of about 0.1 MPa, having a pulse duration of approximately 900 μsec, which is weak and slow, compared to pressure pulses of 44 MPa and pulse durations of about 4 μsec, generated with our device, which additionally generates a negative pressure pulse. Using the same shock tube, Teshima et al. (1995) showed that the destruction of spheroplast of recombinant cells of *E. coli* JM 109/pKPDH2 can be monitored by measuring phenylalanine dehydrogenase activity leaked from the cells. Electron microscopic analysis of the cells after 100 shock waves at 14 MPa showed cell rupture.

It is to be expected that the effectiveness of the shock wave depends on the maximum pressure amplitude (peak compressional and rarefactional), the rise time, the duration of the pulse, and the repetition rate.

The exact mechanism of the induced microorganism death is still unknown. Cavitation, micro jets, acceleration, shearing forces, and formation of free radicals may cause the observed effect. These mechanisms will be affected on the suspension media used.

Another possible mechanism of cell death are resonance effects and collisions between the microorganisms. Experiments using higher microorganism concentrations possibly could help to determine the importance of collisions for microorganism death.

The compression of the suspension causes a transient increase in temperature. Nevertheless in this case the temperature rise is very small. Berens et al. (1989) estimated the temperature rise at the second focus of an electrohydraulic lithotripter to be roughly 0.01°C. This temperature rise depends on the discharge rate. Considering that lithotripters use ellipsoidal reflectors to concentrate the energy generated at the first focus, in this case, where a parabolic reflector was used, the temperature rise is expected to be even lower. Because of this, microorganism death is not associated with an increase in temperature due to the positive pressure pulse. Nevertheless, as already mentioned, localized temperature rise due to collapse of cavitation bubbles may produce microorganism death.

An experiment, that could help to understand the mechanisms involved in microorganism death would be to partially immobilize the microorganisms in gelatin, instead of suspending them in a liquid. This would reduce cavitation and microorganism collisions almost completely, even if it is well known that *E. coli* is capable of moving through gelatin.

For biological tests, the reported variation of K is low.

5 Conclusions

Our results indicate that electrohydraulically generated shock waves are capable of producing a significant reduction in an *E. coli* population. An increase in the applied shock wave number between 500 and 2000 shock waves, generated using an 80 nF capacitor and a voltage of 20 kV, produces a nearly exponential reduction in the *E. coli* population.

A detailed knowledge of microorganism shock wave killing could have many practical applications to the food industry, specially considering that pressures as high as 100 MPa do not denature proteins, which means that this process may be selective. Furthermore, the treatment of pharmaceutical solutions and suspensions as well as biomaterials, could also be possible. This would be of special interest in those cases where microorganism inactivation using radiation, chemical substances or thermal procedures deteriorates important properties or is restricted by law. In terms of food preservation, shock waves give solids a better chance to bacterial survival than liquid or foamy foods.

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