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# English translation(partly)

## Ultrasoon geluid als sterilisatiemethode

## (Ultrasound as sterilization method)



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# Ultrasound as sterilization method

*Literature investigation for the effects of sonication and cavitation on micro-organisms and the application possibilities in the industry*

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**Translation**

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## Summary

Sterilisation, the destruction or deactivation of bacteria, viruses and other micro-organisms, is an essential activity in many industry sectors, in research and in medicine. As a result, much research goes into creating new more effective and more efficient methods. One of the possible methods by which to weaken or kill micro-organisms is called ultrasonication. This is the term used to describe the treatment of a medium with ultrasound.

Sound is made up of a longitudinal wave that creates alternating compression- and expansion phases in the medium that, in turn, produce microscopic bubbles. This is called cavitation. The bubbles that develop as a result of this are also affected by the compression and expansion, meaning that they can be either stable or unstable. Non-inertial cavitation is the development of stable bubbles, and these bubbles shrink and grow more or less equally during compression and expansion. However, micro-bubbles can also be unstable, and this is called inertial cavitation. These bubbles implode fairly quickly, resulting in intensive effects, such as extreme increases in pressure and temperature.

A number of factors influence inertial and non-inertial cavitation. These factors affect the threshold that applies to the formation of bubbles and the effect they have when they implode. Most of these factors have an influence on both these aspects – e.g. they make bubbles develop more easily but also ensure less extreme effects.

Sonication and cavitation cause wide-ranging effects. Extreme pressure and temperature increases, shock waves, chemical reactions and streaming can all take place within the medium.

Sonication and cavitation are extremely effective in the combating of micro-organisms, and are able to damage micro-organisms in various ways. However, the most significant effects are increases in pressure and mechanical stress. These effects can rip apart the membranes of micro-organisms or make them permeable, allowing substances to be diffused into or out of the cells.

Ultrasonication is extremely effective when combined with other sterilisation methods. In addition, the resulting mixing of the medium and the rupturing of membranes increase the effectiveness of antibiotics and other chemical substances.

## 5.4 General effects of sonication and cavitation on micro-organisms

Ultrasonication and cavitation create enormous amount of energy and extreme forces, so it's no surprise that they are able to damage micro-organisms. The way in which they damage or even destroy these micro-organisms has proved to be similar in most cases. However, a number of micro-organisms, such as viruses and spores, are known to be especially resilient and can only effectively be combated in combination with other techniques. The effects of sonication and cavitation are described below.

**Pressure fluctuations**
The enormous pressure increases created by sonication and cavitation disrupt the membranes and increase their permeability. When exposed to sonication, micro-organisms have been found to release internal proteins and molecules without their membranes being ruptured. This became evident when it was observed that, during ultrasonication, a variety of substances were released, such as calcium, nitrogen, acids, glycopeptides, fatty acids, glycolipids and other molecules [Piyasena et al., 2003]. However, missing from this list are phospholipids, the building blocks of all cell membranes. The membranes are thus still intact, although many more molecules are diffused. Water also enters the cell, lowering heat resistance. Besides this, other substances such as antibiotics penetrate the micro-organisms much more effectively. Pressure fluctuations also give rise to the mechanical effects of cavitation, including shock waves, shear stress and micro-streaming [Blume, 2004] [Earnshaw et al., 1995] [Kondo and Kano, 1998] [Oster, 1947] [ Raman and Abbas, 2007].

**Water jets**
As discussed in chapter four, non-symmetric bubbles, which create extremely strong jets of water, develop in the vicinity of solid surfaces during cavitation. These jets have such high velocities that they can even damage steel. This means that they can also damage micro-organisms by penetrating the membranes and breaking down the molecules. This effect is extremely localised and could perhaps be enhanced by suspending relatively large particles in the medium. [Vollmer,1999].

**Mixing**
The effects created by cavitation are most handy, especially in non-homogenous systems. The compound of the medium is mixed, substances are spread more homogenously and aggregates from solid matter, such as sediment, are broken down. This effect is very useful because contact surfaces are increased, meaning that more reactions can take place and more cells can be affected [Suslick, 1989].

**Temperature**
In the past, it was believed that temperature was the most significant factor in the destruction of organisms by means of cavitation. This has proved to not be the case, since hotspots are localised and the heat diffuses rapidly. When the heating of a medium during ultrasonication was disproved by Ananta et al, there was no noteworthy change in the destruction rate of micro-organisms. Despite a lack of extreme heat, the effectiveness of ultrasonication remained the same. Hotspots are therefore not a significant factor in the destruction of micro-organisms. They do kill some micro-organisms, but not nearly enough [Ananta et al., 2005] [Pitt, 2005].

**Electrical current**
Generally, the generation of electrical potential during cavitation is as effective as the heating effects – it is harmful towards micro-organisms, but the potential is very localised and typically has little effect. Nonetheless, this current is important in influencing organic chemical reactions [Gimenez, 1979].

**Forming of radicals**
Radicals, extremely reactive molecules and atoms, are released during the implosion of micro-bubbles. Radicals are lethal towards micro-organisms and are used by e.g. white blood cells to destroy pathogens. They also affect numerous organic chemical reactions. Radicals were thus previously thought to play a significant role in the destruction of micro-organisms. This did, however, not prove to be the case. Not with respect to bacteria and spores in any event. Radicals do play a role, but even without these molecules and atoms, widespread destruction of micro-organisms was still observed. Nonetheless, radicals have an effect on the breaking down of DNA by means of mechanical stress. The resulting fragments are extremely sensitive to radicals and therefore enhance the effect [Ananta et al., 2005] [Earnshaw et al., 1995] [ Furuta, 2004] [Hua, 2000] [Kondo and Kano, 1998] [Piyasena et al. 2003] [Qian et al., 1999].

**Organic chemical reactions**
As previously mentioned, many organic chemical reactions are affected by sonication and cavitation. They can be initiated, slowed down, accelerated or even sent on a completely different trajectory. These effects naturally disrupt the metabolisms of many cells, but do not play a major role in the destruction of cells [Oster, 1947]. It may be possible to enhance the effects by introducing substrates to the medium that will further disrupt metabolism through sonication, although the viability of this is questionable.

## 5.5 Effects on specific micro-organisms

**Bacteria**
Generally speaking, the destruction of bacteria by means of sonication and cavitation is extremely effective.
The destructive effects of ultrasonication and cavitation are a particular result of the mechanical stress on membranes. When the force of this stress becomes greater than the tensile strength of the membranes, they can rupture.
Membranes within cells and surrounding them are essential to the integrity of micro-organisms. If these membranes are irreparably damaged, cells are doomed because their permeability barriers are lost and they are unable to keep any electrochemical gradient around the membranes or maintain their metabolism. Damage to these membranes is thus often the cause of sterilisation through sonication, since destroyed bacteria often display large holes. However, it is not necessarily the cause. Ananat et al. established that the membranes of some of the destroyed bacteria were still intact. The destructive effect probably comes as a result of the membranes becoming more permeable, not necessarily because they are ruptured.
There is a distinct difference in the sensitivity to sonication and cavitation between Gram negative and Gram positive bacteria. It has emerged that E. coli, a Gram negative bacterium, dies twice as fast as the Gram positive L. rahmnosus when exposed to ultrasonication. This difference proves to not necessarily be the result of a much thicker and denser cell wall, but due to the ultrasonication having an extremely strong effect on the membranes of Gram negative bacteria in that the lipopolysaccharide layer of the exterior membrane is disrupted. This is a very positive finding, because this type of bacteria resists most treatments, including antibiotics, and is often extremely pathogenic [Ananta et al., 2005] [Blume and Neis, 2004] [Boucher et al., 1967] [Brambilla, 2006] [Burgos et al., 1972] [Burleson, 1975] [Dahi, 1976] [Deshpande and Prausnitz, 2007] [Earnshaw et al., 1995] [Foladori et al. 2007]
[Furuta, 2004] [Hua, 2000] [Hughes, 1961] [Jyoti and Pandit, 2004] [Qian et al., 1999] [Piyasena et al., 2003] [Scherba et al., 1991] [Vollmer, 1999].

**Spores**
Spores are so resistant towards sonication that this technique is even used to purify suspensions in which spores are actually meant to be present. However, sonication has a damaging effect, making the spores swell up and damaging their exterior surfaces. The swelling is due to pressure fluctuations and mechanical stress that make the spores more permeable. As a result, the spores become much less resistant to other techniques [Burgos et al., 1972] [ Piyasena et al., 2003].

**Animal cells**
It has emerged that larger cells are subject to more severe damage by sonication and cavitation due to their greater reactive surface area. Animal cells, like all eukaryotes, have much in common with prokaryotes and viruses. Animal cells, in contrast to the cells of other organisms, have no cell walls. As a result, damage to the cell membrane probably has an even greater effect, since the cell’s integrity and shape depends solely on this plasma membrane.
Besides the disruption of membranes, DNA damage to eukaryotes is another major factor in the destruction of animal cells. It has been established that many dead cells remain fully intact and can even continue living temporarily, although they cannot reproduce or produce protein and are thus in the process of dying [Kondo and Kano, 1998].

## Plants

The application of ultrasonication to plants is currently creating more and more interest, especially with respect to algae. It has been established that, even following brief treatment, ultrasonication is extremely effective in combating algae. Algae cells float on water by means of vacuoles, microscopic bladders filled with gas. Mechanical stress and pressure fluctuations caused by ultrasonication damage these bladders, making them sink and die [Mahvi et al., 2005].
Vascular plants, just like the other eukaryotes, have large, complex cells and are thus extremely sensitive to ultrasonication. Plants might be even more sensitive because they store water in large, central vacuoles. These bladders are, in the first place, more sensitive to sonication due to the fact that, when damaged, the osmotic potential of the cells is disrupted. Secondly, all manner of substances, including enzymes, ions and even toxic by-products are stored in them. If these substances escape, the consequences for the cell are dire. Finally, these large vacuoles also determine the shape of the cell. Under normal conditions, the cell is pressed up against the cell wall and this cell wall ensures that the cell retains its shape. The vacuole provides the necessary pressure and, when it leaks, the cell loses its shape [Campbell, Reese, 2004].

## Fungi

Although little research has gone into the effects of sonication and cavitation on fungi, it's a known fact that fungi display less growth following treatment with ultrasonication. The mechanics, for the most part, are probably similar to that of bacteria and eukaryotes – damage to the DNA and an increase in the permeability of membranes so that the internal environment of the organism is disrupted [Scherba, et al., 1991].

## Viruses

Many viruses offer extreme resistance to the effects of ultrasonication and cavitation, although envelope viruses in particular are more sensitive. It has emerged that the infectiousness of viruses can clearly be decreased through ultrasonication, more than likely by damage to the viral envelope, a membrane surrounding the virus. This envelope is essential in order for the virus to contaminate host cells, and damage to it deactivates the virus. However, certain viruses are not surrounded by an envelope, but by a capsid. This capsid is much stronger than an envelope, and it has been proven that viruses with capsids are much less affected by ultrasonication [Scherba, et al., 1991].

If virus particles are small enough, they can also be broken apart. It has been shown that particles of the tobacco mosaic virus need to simply be split into multiple fragments. It has emerged that the amount of fragmentation depends on the amount of cavitation applied [Newton, 1951] [Oster, 1947] [Scherba, et al., 1991].

## Argument and conclusion

Ultrasonication and cavitation are methods that are extremely effective in all kinds of applications. They are particularly effective in the combating of micro-organisms. We have discussed the fact that compression and expansion of a medium due to the longitudinal sonication waves causes inertial and non-inertial cavitation. The resulting temporary and stable bubbles result in a wide range of effects, such as extreme pressure- and temperature increases. In addition, there are numerous factors that influence cavitation, giving it the ability to be accurately managed.
Sonication and cavitation have proved to have a wide range of effects on micro-organisms and to be effective sterilisation methods. Particularly pressure increases and mechanical stress on cell membranes and viral envelopes lead to severe damage and a rise in permeability. When used in combination with other techniques, sonication is also very effective as a sterilisation method.
There are, of course, many more imaginable applications for sonication and cavitation. This is especially due to the fact that these processes do not leave behind any chemical substances and, if carefully controlled, will hardly affect solid materials. This means that ultrasonication is ideal for applications within the foodstuffs industry, where products must be effectively sterilised without taste or texture being affected.
The fact that the medium is mixed makes this technique extremely suitable for the cleansing of waste water and water lines. These in particular contain a wide range of waste, such as high contaminant concentrates, sediment, particles and other aggregates. These are broken down and mixed into the medium, allowing for better circulation and enabling the more effective combating of micro-organisms by means of other methods.
In addition, these techniques can be applied to great effect in the medical- or technology industries, in which instruments and aids, such as scalpels, prostheses and even microchips must be purified without being affected by corrosion. Biofilms, in particular, are often a problem. These are thick and resilient colonies of bacteria. Sonication and cavitation are extremely effective in breaking these down and loosening them.
Cavitation is also employed in the in vivo treatment of kidney stones, for instance, and research is being performed into promoting the protein intake of cells.
Finally, sonication and cavitation also features in specific biomolecular research that ensures that cells can be transfected with DNA with greater ease.
There are, however, a few problems with sonication and cavitation. Firstly, they are difficult to apply on a large scale. The fact that cavitation is most effective if the medium is treated homogenously with ultrasound makes it difficult to apply it in reactors. Secondly, this is a technique that uses large amounts of energy. Lastly, its exact effects on a large number of micro-organisms are still partly unclear. It is, however, a promising technique because it is influenced by so many factors, As a result, it can be accurately managed and no chemical substances remain behind in the medium. Ultrasonication has proved to be extremely effective in combating certain resilient micro-organisms, especially in combination with other treatment methods.

Ultrasonication and cavitation are promising methods with numerous resulting effects and application possibilities. However, before it can be applied on a large scale, much research is still required into treatment methods, optimum conditions and its effects on micro-organisms.