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Feasibility study of ultrasound as water disinfection technology

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ABSTRACT

This article presents a section of project work related to the use of ultrasound technology as an eco-friendly water disinfection process. Scientific and economical evaluations are presented for two different ultrasound systems. The effects of ultrasound frequencies (20, 279 and 817 kHz), amplitude (acoustic power) and the treatment time for bacteria survival were studied. Experiments performed on a laboratory scale using two biological indicator micro-organisms *Bacillus subtilis* and *Escherichia coli* indicated that disinfection efficiency is affected by bacteria morphology, ultrasound frequencies and energy densities entering the system. As the spore-forming bacteria *B. subtilis* seemed less vulnerable to ultrasound exposure, a significant *E. coli* inhibition of 2.97 ± 0.58 log was achieved in 5 min treatment time at 817 kHz.

Keywords: Ultrasound; Disinfection; Microbiology; *Escherichia coli*; *Bacillus subtilis*

1. Introduction

Disinfection is an indispensable and significant part of water treatment systems that enable safe drinking water usage or safe wastewater reusing and recycling. Chlorination (mainly in the hypochlorous acid form HClO) has been the preferred water disinfection technology despite evidence concerning its relationship to the formations of hazardous disinfection by-products (DBPs), such as trihalomethanes (THMs) and haloacetic acids. DBPs have been identified as potential human carcinogens and harmful for the environment even at low concentrations of less than 0.1 mg/L [1,2]. Other disadvantages of this process are related to its ineffectiveness against protozoa such as *Cryptosporidium* and *Giardia* and, due to the need for an additional de-chlorination step, an increase in disinfection costs of 20–30% [3].

Despite the above statements, chlorination is still the advanced disinfection technique applied today in more than 80% of cases.

Ozone is a strong oxidising agent (stronger than chlorine), effective at destroying bacteria (it is primarily responsible for *Escherichia coli* inactivation in drinking water) [4] and viruses [5,6], as well as cyst-forming protozoan parasites such as *Giardia* and *Cryptosporidium*, which are particularly resistant to most other disinfectants [7].

The disadvantages of using ozone mainly relate to the expensive investment and maintenance costs during ozone production (it has to be produced on-site from air or pure oxygen with the help of electrical field), low stability of the ozone and consequently fast decomposition. Ozonation represents no risk to the

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formation of halogenated compounds unless in the presence of bromide in water [8].

The more commonly used alternative to chlorination is ultraviolet irradiation, having a comparable and often more effective disinfection efficiency for viruses and bacteria [9]. The success of UV technology is largely attributable to low costs, as well as the absence of toxic by-products [10]. However, suspended solids in water hinder UV light transmission through the water and, as a consequence, reduce disinfection efficiency.

An array of new challenges exists including the treating of resistant pathogens (*Giardia*, *Cryptosporidium*), minimising the chemical loads and DBPs' formation, and therefore stringent environmental and safety regulations have led into intensive research and the developments of alternative disinfection methods. Amongst others, the more important concerns relate to the fact that some micro-organisms are becoming resistant to existing disinfection techniques involving biocides, ultraviolet irradiation and heat treatment. As an alternative to classical chemical disinfection technologies, radiofrequency waves, pulsed electric field and ultrasound are coming to the fore [11,12].

The damaging effects of ultrasound on micro-organisms were recognised quite early on. According to theory, the disinfection capacity of sonochemical treatment is due to the phenomenon of acoustic cavitation, i.e. the formation and collapse of bubbles filled with gas (micro-bubbles). Each micro-bubble acts as a local micro-reactor producing extreme temperatures (1.0^{10} K/s) and pressure gradients (1.01325×10^5 kPa). These elevated temperatures and pressures, high velocity water jets and free radicals formed from water decomposition can be directly exploited for microbial inactivation [13].

In general, ultrasound irradiation has been proved to be effective for the inactivation of bacteria such as total coliforms, faecal coliforms and *Pseudomonas* spp. [14], *E. coli* [15], *Microcystis aeruginosa* [16,17] and bacteriophages such as ΦX174 and MS2 [18]. The inactivation efficiency rate is influenced by several parameters related to the ultrasound frequencies, and the intensities and "types" of micro-organisms to be treated. Drakopoulou et al. observed higher resistance of Gram-positive bacteria such as *Clostridium perfringens* and faecal streptococci in comparison to Gram-negative bacteria such as total coliforms, faecal coliforms and *Pseudomonas* spp. under 24 kHz ultrasound treatment [14].

A combination of ultrasound with other disinfection techniques (ozone, UV and chlorination) might lead to synergistic effects resulting in better microbial inactivation efficiency and in the reduction of disinfection agents' consumptions [19–21].

The overall objective of the presented work was to examine the disinfection efficiency of ultrasound within the range from low frequency/high intensity to high frequency/low intensity. Two different types of ultrasound equipment, three different emitter accessories and two biological indicator micro-organisms, namely *Bacillus subtilis* and *E. coli K12*, were used during the study. Parameters such as ultrasound frequency, intensity and density that affect the reduction in bacteria are discussed in detail. Cost efficiency evaluation, scaling-up feasibility and comparison with other disinfection processes are also discussed.

2. Materials and methods

2.1. Testing micro-organisms

Bacillus is a genus of Gram-positive, rod-shaped bacteria with several species. They can be obligate aerobes or facultative anaerobes, ubiquitous in nature. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. *B. subtilis* is an important "model" micro-organism and is used as an indicator during the validation procedures of UV disinfection reactors. Experiments were performed on non-pathogenic strains in the lyophilised forms of *B. subtilis* spores.

E. coli is a Gram-negative, rod-shaped, non-sporulating bacterium commonly found in the intestines of mammals. It is frequently used as a "model micro-organism" in microbiology studies. A non-pathogenic strain of *E. coli K12* in lyophilised form was used during the research.

2.2. Ultrasound equipment

Two different ultrasound systems were used: the low frequency (20 kHz) probe type from Sonics&Materials with two different accessories (tapered micro-tip and replaceable tip) as presented in Fig. 1 and the high-frequency plate type from ELAC Nautik, operating at two different frequencies, namely 279 kHz and 817 kHz (Fig. 2).

2.3. Experimental procedures

2.3.1. Characterisation of ultrasound systems

Acoustic power entering the system and sonochemically oxidative species formation rate was defined according to the experimental procedures described in Vajnhandl et al. [22].

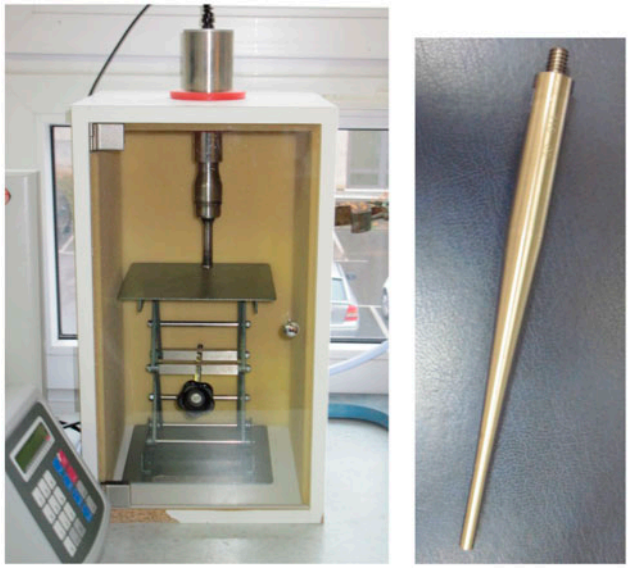

Fig. 1. 20 kHz probe ultrasound system with replaceable tip (left) and tapered micro-tip (right).

Fig. 2. High-frequency (279/817 kHz) plate ultrasound system.

The characteristics of the ultrasound systems used in the study are presented in Table 1.

2.3.2. Microbiological monitoring

In order to simplify microbiological monitoring, it is a widespread practice to monitor so-called indicator organisms in water samples instead of all the possible pathogens. By this monitoring strategy, microbial analysis is easy to perform as suitable cultivation media or easy rapid tests for the targeted bacteria are available (e.g. Colilert, IDEXX). Cultivation-based monitoring is suitable for the analyses of known pathogens and indicator organisms that are thought to represent a certain "trend" in a monitoring area.

The more monitored indicator organisms are *E. coli* and coliform bacteria. The microbiological qualities of drinking water and other types of water are assessed by analysing these bacteria.

So far, the abundance of certain micro-organisms has been mainly monitored by cultivating sample materials on different media and the resulting frequencies and numbers of grown bacteria are given as colony-forming units (CFU)/sample volume.

The Plate count method was used for enumerating *B. subtilis* bacteria according to the standard methods for coliforms' bacteria and *E. coli* ISO 6222:1999 [23]. A stock solution was prepared by the inoculation of *B. subtilis* spores in powder (corresponds to approx 1.0^{11} spores) in 100 mL of sterile distilled water at a temperature of 5 ± 3°C. The concentration of *B. subtilis* spores in suspension corresponded to 10^9/mL. Solutions for various treatments were prepared by the addition of 1 mL suspension to the 1 L of sterile distilled water. The concentration of spores in this solution corresponds to the 10^6 /mL.

In order to prepare a reference sample, 1 mL of solution with a known theoretical concentration of *B. subtilis* was transferred using a sterile pipette on sterile petri plates. Fifteen millilitres of nutritious solutions were added. The agar (yeast extract agar Oxoid) and the sample were gently mixed immediately by moving the plates in figure-eight motions. After the pour plates had cooled and the agar had hardened, they were inverted and incubated at 36°C for 48 h (±4 h). At the end of the incubation period, we calculated the number of CFU per volume of sample. The average concentration of *B. subtilis* was defined according to the following equation:

$$[\text{conc.}] = \frac{\sum \text{number of colony on countable boxes}}{1 + \sum \text{dilutions}} \times \frac{1}{\text{first countable dilution}} \quad (1)$$

With regard to the qualitative and quantitative determinations of *E. coli*, Colilert-18 is the most appropriate method, which is quick, simple and reliable. For *E. coli* determination, we used Quanti-Tray technology (IDEXX Laboratories Inc, Westbrook, ME, USA) with

Table 1
Ultrasounds characteristics

Frequency (kHz)	Probe area (cm^2)	Acoustic power (W)	Intensity ($W\,cm^{-2}$)	Density ($W\,mL^{-1}$)
20[a]	1.3	50	38.5	0.5 (0.42)
20[a]	1.3	65	50	0.65 (0.54)
20[b]	0.07	20	285.7	0.2 (0.17)
279	25	150	6	1.5
817	25	160	6.4	1.6

[a]Replaceable tip.
[b]Tapered micro-tip.

corresponding nutritious solutions according to the manufacturer's instructions.

In order to create a stock solution contaminated with *E. coli K12*, a 0.2 g (tablet) was introduced into 1 L of sterile distilled water. The theoretical concentration for the doping of sterile water was 25,000 *E. coli*/100 mL. All experiments were performed at sample volumes of 120 mL.

The sample and substrate were mixed and incubated for 18–22 h at 37 ± 1°C. The *E. coli*-positive wells were yellow and fluoresced under UV light (365 nm). By means of a table provided with the system, counts of the number of positive wells were transferred to the most probable number (MPN) of the target organisms.

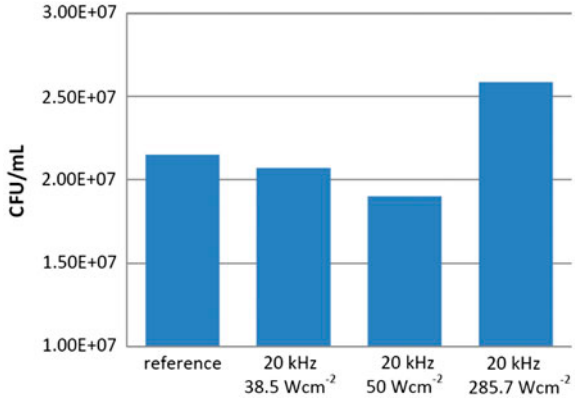

Fig. 3. Influence of ultrasound intensity on CFU/mL reduction of *B. subtilis* in comparison to the reference for the frequency 20 kHz.

3. Results and discussion

3.1. B. subtilis

The results of *B. subtilis* treatment with low-frequency ultrasound (replaceable tip and micro-tip) are presented in Fig. 3.

The obtained results indicate an insignificant inhibition effect of ultrasound treatment at 20 kHz on *B. subtilis* spores for different ultrasound intensities and 60-min contact time. The initial average concentration of spores corresponded to 20.43×10^6 CFU/mL and average concentration after 60 min of the ultrasound treatment, i.e. with micro-tip, corresponded to 25.86×10^6 CFU/mL. In fact, the concentration of spores after the treatment even increased.

The increase in concentration of the *B. subtilis* spores under the ultrasound treatment has been attributed by some authors to the de-clumping effect [24]. *B. subtilis*, as well as many other bacteria, can agglomerate within spherical clusters. Ultrasound action provides an initial rise in cell numbers as a result of de-clumping. Clusters are difficult to destroy using classical disinfection (such as chlorination) because it destroys the micro-organisms on the surface but often leaves the innermost intact. It is believed that ultrasound through physical and chemical effects arising from acoustic cavitation can de-agglomerate bacterial clusters and the dispersion of single cells, and in such a way make them more vulnerable to other disinfection agents.

In the case of *B. subtilis* treatment, there was no clear correlation between ultrasound intensity (Table 1) and disinfection efficiency. According to the theory of ultrasound, a significant CFU reduction in *B. subtilis* was expected in the case of using a tapered micro-tip where ultrasound intensity was six orders higher, as it was in the case of a replaceable micro-tip.

The effect of ultrasound treatment at higher frequencies and lower ultrasound intensities indicated a slightly different picture (Fig. 4). Anyhow, there was an insignificant difference in reduction efficiency when comparing both frequencies 279 kHz and 817 kHz. The results suggested a higher frequency favourite *B. subtilis* spores' reduction, but in both cases the reduction efficiency was insignificant if we took into account the 60 min treatment time.

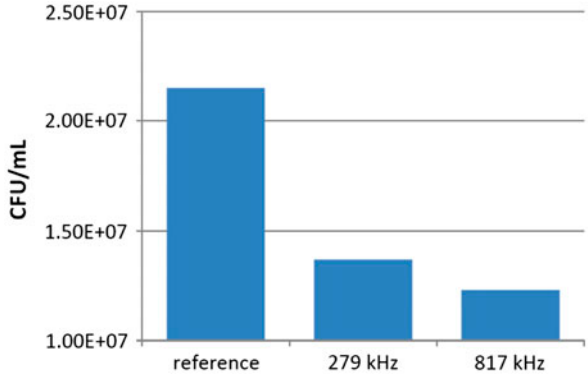

Fig. 4. Influences of higher frequencies 279 kHz and 817 kHz on the CFU/mL reduction of *B. subtilis* in comparison to the reference.

In the case of highly resistant spore-forming microorganisms, it is very difficult to discuss as to which operating parameter of ultrasound is the key element in the destruction mechanism. Some authors suggest that spores are resistant even to sonolytically formed H_2O_2 because of the spore's coat, which serves as a barrier for the diffusion of H_2O_2, or the coat proteins, which oxidise it before H_2O_2 reaches the spore core [25].

3.2. E. coli K12

In the case of *E. coli*, the ultrasound frequency displayed a strong influence on the inactivation and increase in frequency from 20 to 817 kHz, as presented in Fig. 5.

In contrast to *B. subtilis*, a significant inhibition was evident even for low frequency 20 kHz and up to a six times shorter contact time. *E. coli* inactivation most likely resulted from a combination of physical and chemical mechanisms during acoustic cavitation. The roles of chemical mechanisms during bacteria inactivation processes could be explained by the hydrogen

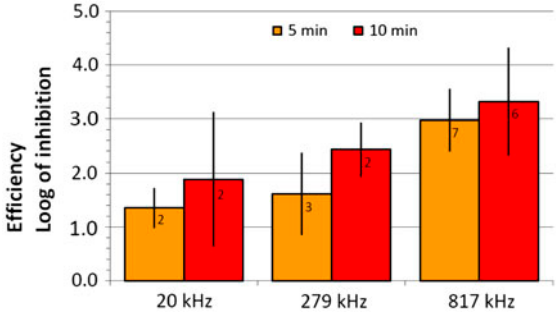

Fig. 5. Inhibition of *E. coli* as a function of applied frequency.

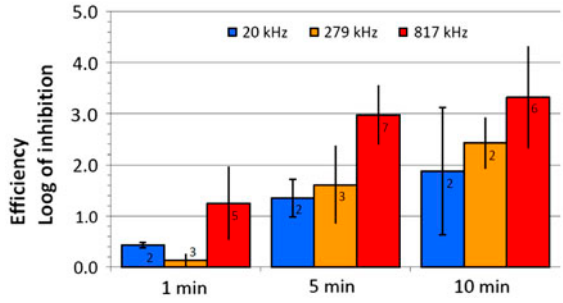

Fig. 6. Inhibition of *E. coli* as a function of treatment time.

peroxide formation rates at stated frequencies. Hydrogen peroxide was formed after recombining the very reactive hydroxyl radicals during sonolysis and exhibited zero-order kinetics. The correlation between the hydrogen peroxide formation rate, frequency and acoustic power entering the ultrasound system was reported previously [22]. An excerpt of our previous research work is presented in Table 2.

Thus, it appears that the disinfection effect was optimally performed within the same frequency range as optimal hydrogen peroxide production.

On the other hand, the concentration of formed hydrogen peroxide was quite low (in μmol/L), so it is hard for it to be the leading destroying mechanism. Moreover, aerobes such as *E. coli* employ a number of mechanisms, including peroxidase and catalase, for destroying hydrogen peroxide, a very well-known bactericide [26].

Other possible mechanisms include direct free-radical attack, followed by physical disruption of cell membranes. Physical effects, as a consequence of micro-bubbles' implosion, sheared a cell membrane so the chemical oxidants can easily enter the cell.

Also, a significant aspect of destroying bacteria cells could be a temperature rise during sonochemical treatment. A notable rise in bulk solution temperature was detected, especially in the case of low-frequency high-power ultrasound. The initial temperature of 25°C increased up to 50°C over 60 min of sonication.

The highest inhibition efficiency was observed for 817 kHz ultrasound regardless of treatment time. At 5-min contact time, the concentration of *E. coli* decreased by 2.97 ± 0.58 log in demineralised water. The inhibition at 10-min contact time was quite comparable.

3.3. Energy and cost-efficiency evaluation

Studies comparing the operational costs associated with disinfection methods showed that the operational

Table 2
H_2O_2 formation rate for stated ultrasound conditions

Frequency (kHz)	279			817		
Acoustic power (W)	50	100	150	50	100	150
Formation rate (μmol L^{-1})	1.68	3.45	5.16	1.95	3.66	6.79
R^2	0.998	0.998	0.993	0.989	0.998	0.993

cost of treatment using ultrasound technology was still two orders of magnitude higher as compared to chlorination [27]. Despite evident *E. coli* inhibition over very short contact time, scaling up this technology for disinfection purposes seemed rather unrealistic, mainly due to the following facts.

One of the reasons was the inefficient conversion of electrical energy into the desired mechanical (cavitation) energy. Conversion rates for ultrasound equipment were determined by calorimetric measurements and have been published previously [22]. A maximum 41% conversion rate of electrical to acoustic energy was achieved for the frequency 817 kHz (380 W input electric power). At 20 kHz, this conversion rate was even less favourable. This means a lot of energy was lost to undesirable effects such as heating, which in our case could have represented an additional destructive mechanism. Jyoti and Pandit [19] calculated the cost of the treatment using ultrasonic horn and ultrasonic bath when neglecting the incomplete conversion of electrical to mechanical energy. As was observed from our previous experiments, this conversion is very variable amongst different types of ultrasound equipment and could significantly affect cost-efficiency evaluation.

In ultrasound technology, a very large amount of energy is usually needed for treating very small reaction volumes. When scaled up to industrial size, this represents huge amounts of energy requirements that need very high energy capacity equipment. This results in very high capital cost of equipment and high operating and maintenance costs. According to Mahamuni and Adewuyi, the energy density, in order to make the process economically viable on an industrial scale, should not be more than 0.05 W/mL [28]. For the experimental conditions used in this study, the energy density was within the range from 0.2 to 1.6 W/mL (Table 1). At higher frequencies, the ratio between energy consumption and treated volume (expressed as energy density) becomes less favourable in terms of cost efficiency.

4. Conclusions

Efficiency and applicability of ultrasound technology was tested for water disinfection purposes using two different biological indicator micro-organisms. Ultrasound assisted inhibition studies indicated that disinfection efficiency under applied experimental conditions depends on micro-organism (non-sporulating or sporulating bacteria). Differently applied ultrasound frequencies and intensities did not exhibit any inhibition effect on *B. subtilis* spores, proving their efficient resistance.

In contrast to non-sporulating bacteria, an evident level of *E. coli* inactivation can be achieved within the first few minutes of treatment. The most significant inhibition was achieved at 817 kHz. In the case of *E. coli* inhibition, some correlation between ultrasound process parameters (frequency and H_2O_2 formation rate) and bacteria reduction was evident.

Momentarily economic evaluation makes ultrasound technology less attractive, but this technology could find its place in water disinfection also because of increasing environmental concerns and awareness that the use of toxic biocides should be replaced or reduced.

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