# SHORT REVIEW OF RAW WATER DISINFECTION METHODS WITH FOCUS ON ULTRASONIC SYSTEMS

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**Abstract**

*Potable water is water of acceptable quality in terms of physical, chemical, and microbiological parameters for safe drinking. Although it is a basic human need and right, ensuring access to drinking water has been and continues to be a challenge. Waterborne diseases are still an issue in many developing countries and deaths from diseases such as cholera, typhoid and diarrhoea remain high. Their prevention and control through protection of water sources and proper treatment techniques are of great importance. Disinfection of water is an important step in water treatment and can be achieved by chemical, physical or hybrid methods. Chemical processes include treatment with chlorine, chlorine dioxide, chloramines, ozone, and hydrogen peroxide. Physical methods include disinfection with heat, UV irradiation, and ultrasound. New methods are being developed and studied to advance the disinfection process. This paper provides a brief overview of the most commonly used disinfection methods and a case study on ultrasonic disinfection of raw (surface) water. The disinfection efficiency of a horn probe (20 kHz) and an ultrasonic bath (25/45 kHz) under different operating conditions is investigated. TECTA™ pathogen detection system was used for enumeration of Escherichia coli and total coliform colonies. For a volume of 200 mL, complete inactivation of E. coli is observed after less than 10 minutes of exposure time in a horn probe setup. For larger volumes (1000 mL), the bath operating at 25 kHz provided better disinfection for the same period of time. In general, the smaller the volume and/or the longer the treatment, the higher the disinfection rate for microorganisms. In addition, a "de-clumping" effect was observed in all experiments.*

**Keywords:** *potable water, disinfection, ultrasound, Escherichia coli, total coliforms*

## 1. INTRODUCTION

Water is a basic human need, yet only about 1% of the water on our planet is available for direct use. Moreover, available water often needs to be adequately treated to be safe for human consumption [1]. Drinking or potable water is water of acceptable quality in terms of physical, chemical and microbiological parameters for safe drinking and cooking [2]. According to the World Health Organisation drinking water “does not represent any significant risk to health over a lifetime consumption, including different sensitivities that may occur between life stages” [3]. In addition to various organic and inorganic chemical pollutants, aquatic systems contain many various microorganisms, including potential pathogens. Although mortality rates associated with waterborne diseases have decreased dramatically since the introduction of disinfection as a continuous process in water treatment, the number of deaths from diseases such as cholera, typhoid and diarrhea is still high in developing countries [4]. Generally, the greatest microbial risks are in association with consumption of faecal contaminated water [3]. To assess microbiological contamination, it is necessary to find an indicator microorganism for routine water analysis. Characteristics of the indicator organism are ease of isolation and enumeration, presence in large quantities in normal fecal matter, better resistance to disinfection than pathogens, absence from other bacterial sources that are in contact with water. If the indicator organism is present, fecal contamination and the presence of pathogens is possible [2,3]. Although not perfect, coliform bacteria and Escherichia coli, in particular, fit the criteria quite well. *E. coli* is the most widely used indicator of faecal contamination of water. Alternative choices include counting the thermotolerant coliform bacteria and total coliform counts. Total coliforms include *E. coli*, *Enterobacter*, *Citrobacter*, and *Klebsiella*. Distinguishing characteristic of these microorganisms is their ability to ferment lactose. Some coliforms may originate from sources other than faeces and can survive and grow on certain materials. For this reason, the presence of a small number of these organisms,

especially in untreated groundwater, does not necessarily mean that faecal contamination is present [1-3]. In accordance with WHO guidelines and Croatian national legislation, water for human consumption must not contain any *E. coli* colonies in the entire 100 milliliters of the sample [5].

Disinfection of water is a key step in water treatment for safe water consumption and public health protection. All surface waters and groundwater prone to faecal contamination should be disinfected. Also, residual disinfection is applied in order to prevent microbial growth and contamination in the distribution system [3]. Different chemical, physical and combined or hybrid techniques are used for water disinfection. In order to enhance the efficiency and minimize possible negative effects of disinfection, new techniques are constantly being investigated and developed. Chemical disinfection methods are based on the direct addition of various chemical disinfectants such as chlorine and chlorine compounds, hydrogen peroxide, iodine, and silver salts to the treated water [6]. Ozonation is, also, a chemical method [7,8]. Lately, due to the problems with disinfection by-products formation, physical methods are gaining more attention. Conventional physical methods include filtration, heating (pasteurisation) and ultraviolet radiation [7]. Photocatalysis, cavitation and electrochemical disinfection methods have also been utilised for disinfection purposes [7,9]. Disinfection efficiency depends on the ability of the disinfectant to oxidize or rupture the bacterial cell wall and/or to penetrate the cell and disrupt the cellular activity [10]. The choice of the appropriate technique depends on many factors; the properties of the treated water, the efficiency required, the risk of disinfection by-products, the applicability to large scales and, of course, the associated costs [7]. The most commonly used disinfection methods are described and compared in this paper. In addition, a case study of ultrasonic disinfection of raw water in two different systems is presented.

## 2. DISINFECTION METHODS

Effective disinfection of drinking water plays a crucial role in preventing waterborne diseases and thus ensuring public health [11]. Numerous disinfection methods have been developed throughout history. One of the oldest disinfection methods is boiling (pasteurisation). It is very simple and does not require any chemicals, but it does require a lot of energy, which makes it economically and environmentally unsustainable [2]. Another simple and effective method of eliminating microorganisms from water is filtration. Since it only removes cells from water and does not kill microorganisms, filtration is not a disinfection method, strictly speaking [7]. Many chemicals, such as chlorine, iodine, bromine, silver, potassium permanganate and hydrogen peroxide, are effective disinfectants and can be used for inactivation of microorganisms [12]. Disinfection by chlorine and chlorine based compounds is, by far, the most widely used method for ensuring potable water free of potential pathogens. However, the potentially harmful effects of this method have led to an increase in research and development of other, safer, methods. In recent years, research has been focused on cavitation, electrochemical methods, photocatalysis and disinfection using solar energy [7]. The latter is especially suitable for low-income communities in developing countries due to its low cost. A comparison of some chemical methods for 99% inactivation of *E. coli* is presented in Table 1. Table 3 summarises the main advantages and limitations of mentioned disinfection methods.

### *2.1. Chlorination, clorine dioxide and other chlorine compounds*

The most popular disinfection method, which has been used for more than 100 years, is chlorination. It is based on the introduction of chlorine gas or other chlorine-containing compounds such as sodium hypochlorite or calcium hypochlorite into the water. This results in formation of hypochlorous acid and hypochlorite ions that are capable of oxidizing microorganisms [7]. Chlorine acts as an oxidant and can help with colour and odour control, iron and manganese removal, improvement of coagulation and filtration processes, and degradation of hydrogen sulphide. It also has excellent biocidal properties and prevents algae growth. The biggest advantage of chlorine treatment, besides its high effectiveness in inactivating bacteria and viruses, is its simplicity and low cost, regardless of the size of the system. In addition, chlorine residual provides protection against microbial recontamination throughout the distribution system [13]. However, the major disadvantage is the formation of disinfection by-products (DBPs), which were discovered in the early 1970s [14]. In water, free chlorine and bromide react with

natural organic matter to form halogenated organic by-products such as trihalomethanes, haloacetic acids and haloacetonitriles. Oxidation of organic compounds in water can also lead to the formation of non-halogenated disinfection by-products such as aldehydes and nitrosoamines. Brominated organic by-products, such as bromoform or bromopircine, can be formed after bromide ions are oxidized by chlorine to hypobromate ions or hypobromous acid [8]. Trihalomethanes, among other disinfection by-products, are potentially hazardous to health as they are considered carcinogenic and mutagenic [15]. Other potential negative effects of chlorine include taste and odour problems depending on water quality and chlorine dosage, alteration of redox state, and impairment of coagulation/filtration of dissolved organic compounds. In addition, chlorine treatment is less effective at high pH and against protozoa. Due to the corrosive and oxidising properties of chlorine, special storage and careful handling is required.[2,7,8]

Chlorine dioxide is more effective than chlorine for viruses and protozoa. It is a stronger oxidant, and its disinfection ability is less affected by pH value of the water. Furthermore, it oxidizes iron, manganese and sulphides while encouraging the clarification process [8]. In the absence of excess chlorine this process will not result in formation of halogenated DBPs, but chlorite and chlorate will form. Although chlorine dioxide provides residual activity, it can cause taste and odour problems. Also, this method requires on-site production of chlorine dioxide due to its instability [16].

Chloramination treatment is based on the reaction of ammonia and chlorine leading to the formation of monochloramine. This reaction must occur “in-situ”. It is significantly less effective than chlorination and it is rarely used alone for primary disinfection [7].

*2.2. Ozonation*

Ozone is used in water treatment as a primary disinfectant and oxidant. It is one of the most effective germicides and more effective than chlorine in inactivating viruses and protozoa [16]. In the pH range between 6 and 9, the effectiveness of ozone is not altered. Higher temperatures lead to higher efficiency of the process. It can oxidize iron, manganese and sulphides and control colour, taste and odour [8]. Disinfection by ozone, like chlorination, can produce by-products. While ozone itself does not form halogenated DBPs, the presence of bromide or chlorine can lead to their formation. Other by-products include organic acids and aldehydes. The major disadvantage of ozonation is the high initial cost of the equipment and the need for very energy intensive on-site ozone generation. Ozone is generated by passing dry air or oxygen through a system of high-voltage electrodes [1]. Safety precautions are required, as ozone is very corrosive and toxic.

**Table 1.** Concentration and contact time of chemical disinfectants needed for 99% inactivation of *E. coli.*

| Disinfectant | Concentration, mg/L | Contact time, min | pH | T, °C | References |
|---|---|---|---|---|---|
| **Hypochlorous acid** | 0.1 | 0.4 | 6.0 | 5 | [17] |
| **Hypochlorite ion** | 1.0 | 0.92 | 10 | 5 | [17] |
| **Monochloramine** | 1.0 | 175 | 9 | 15 | [18] |
| **Chlorine dioxide** | 0.25 | 0.68 | 6.5 | 20 | [19] |
| | 0.75 | 0.15 | 6.5 | 32 | |
| **Ozone** | 0.07 | 0.083 | 7.2 | 1 | [20] |
| | 0.0125 | 0.33 | 7.0 | 12 | |

*2.3. UV irradiaton*

A suitable intensity and duration of UV radiation can also be used as a primary disinfectant [16]. It is effective against bacteria and viruses at lower dose, while higher dose is needed for protozoa. UV dose is the product of UV intensity and contact time. Most commonly used dose is 40 mJ/cm-2 [16]. For 99%

inactivation of *E. coli* a dose of 360 μJ/cm$^2$ proved to be lethal [12]. Usually, UV disinfection is performed by passing the water through tubes lined with UV lamps using a wavelength of light around 254 nm. The germicidal effect is achieved by direct effect of UV light on microorganisms' DNA, causing inability to grow and replicate. By-products generated by this method are minimal, it has no taste and odour problems and has no risk of overdosing. However, it may not be an effective method for waters with high turbidity, iron and calcium content [8]. Efficiency is, also, diminished if bacteria cells form flocs or clusters [21]. In addition, it is found that damage caused to the bacterial DNA by UV irradiation can be reversible [21]. Unlike, chemical treatment, UV radiation does not provide protection against biological recontamination in the distribution network. Due to the UV reactor design, chemical fouling and biological film formation are possible, which can contribute to high maintenance expenses [2]. Operational costs are also high, because of need for constant electricity supply. Safe disposal of used UV lamps is a problem, due to mercury which is hazardous.

*2.4. Electrochemical methods*

An environmentally friendly and effective alternative disinfection method is electrochemical disinfection [11]. In electrochemical disinfection methods microorganisms are inactivated by an electric current passed through the water using suitable electrodes. For this purpose, at least one anode and one cathode are immersed into the treated water and connected to the power supply. Although various complex set-ups have been utilised for electrodisinfection, most practical applications are based on simple undivided reactors with monopolar electrode stacks in parallel arrangement [22]. Oxidizing species are generated on the electrodes, and they act as disinfecting agents. They are produced either from the water itself (hydroxyl radicals, ozone) or from species dissolved in the water (chlorine) [22, 23]. When the treated water has a sufficient concentration of chloride precursors, chlorine-based systems are usually used. In this process, chlorine is electrochemically generated on the electrode surface, afterwards it diffuses into the solution where it hydrolyses and hydrochloric and hypochlorous acid are generated [23]. For waters with low chloride concentration, ozone electro generation from water is possible by using high oxygen overvoltage anodes, high current densities, and low water temperatures. Additional option is disinfection by cathodically generated hydrogen peroxide [22]. The generation of oxidants is influenced by various parameters, such as water quality and chemical composition, type of electrolytic process, applied voltage or electric current, pH, temperature, added electrolytes and electrode material [24-27]. The anode material is a very important parameter in electrochemical disinfection and various materials such as different metals, carbon electrodes, mixed metal oxides and boron-doped films have been tested [28]. Gholami [27] used a simple two steel electrodes set-up and achieved complete inactivation of *E. coli* in 5 minutes of treatment time at an applied voltage of 4.5 V and 500 mA current. Other mechanisms contributing to electrodisinfection effectiveness, besides the effect of produced oxidants on the bacteria, are irreversible permeabilization of cell membranes, electrochemical oxidation of vital cell components and electrosorption of negatively charged *E. coli* cells to the anode surface and electron transfer reaction [29]. The advantages of electrodisinfection are associated with the fact that no additional chemicals are required. This eliminates transport, storage, and disinfectant dosage issues. In addition, electrochemical disinfection shows residual effect due to disinfectant generation. Although, a power supply is needed for this technology, renewable energy sources, such as photovoltaic cells, are available, allowing its implementation in locations far from the electrical grid [22,23].

*2.5. Ultrasonic disinfection*

Ultrasound is a longitudinal wave with a frequency range between sonic and mega sonic region (20-600 kHz) [30]. Ultrasonic waves transmit energy through the vibration of molecules in the environment in which the wave is being spread [31]. Ultrasonic devices, usually, operate at frequencies from 20kHz to 10 MHz. High power ultrasound at low frequencies (<100 kHz) has the ability to cause cavitation [32]. Cavitation is a rapid physical phenomenon caused by sudden drop in pressure. It starts with formation of small vapour bubbles inside a liquid medium [33]. Pressure changes cause bubble propagation and eventually a violent collapse. The energy released from the bubble collapse causes extreme local increases in temperature and pressure. "Hot spots", with temperatures over 1000 K, can form in the centre of the collapsing bubble, leading to formation of highly reactive hydroxyl radicals [34]. Pressure

shocks can be as high as several 100 MPa [35], and in the case of asymmetric collapse, "micro jets" can form [36]. Cavitation can be hydrodynamic or acoustic, depending on the local pressure drop mechanisms. In acoustic cavitation, the propagation of acoustic (ultrasonic) waves causes pressure drops and generate cavitation [21].

There are different types of ultrasonic devices available. Most commonly, ultrasonic waves are generated by the inverse piezoelectric effect. Piezoelectric crystals are used to convert high frequency electrical energy to mechanical vibrations. The vibrating part can be made in different forms and different ultrasonic devices are commercially available. The two most used are ultrasonic baths and probes. The radial probe, also called horn probe or sonotrade is a common ultrasonic device that works by submerging the probe into the treated liquid [37]. It produces high intensity cavitation because all of the acoustic energy is transmitted through a small area. In ultrasonic baths transducers are attached to the side or bottom of the tank and this cavitation is considered low intensity due to the large surface area through which the energy is transmitted. In ultrasonic baths, sometimes the bubbles do not actually collapse, but oscillate for many sonic cycles. This means that the extreme conditions caused by bubble collapse do not happen, but micro eddies occur causing shear stress. This type of cavitation is named "stable cavitation" and cavitation with bubble collapse is called "transient cavitation" [21]. For large volumes the ultrasonic flow cell is commonly used. These can generate high ultrasonic intensities while operating in continuous mode [1].

The first attempts to inactivate bacteria with ultrasound date back to the late 1920s [38], and the first explanations of the mechanism are reported in the 1960s [39]. There are a number of theories about the mechanism of ultrasonic biocidal effects [40]. It is commonly speculated that the main effect responsible for bacterial inactivation with low frequency high-power ultrasound is the result of acoustic cavitation [41-43]. The disinfection effect of ultrasound is achieved by the chemical attack of sono-generated hydroxyl radicals, cell death due to localized high pressures and temperatures, and cell damage caused by the shear forces. The rupture of bacterial cell walls allows chemical oxidants to diffuse into the cell and disrupt intracell structures of the microorganism [31,44].

Advantages of ultrasonic disinfection include simple and flexible design with low capital costs, easy and effective coupling with conventional treatments, high inactivation efficiency for certain microorganisms, and oxidation of natural organic matter without disinfection by-products formation. Downsides of this technology include high energy demand, high maintenance and operational cost, increase in water turbidity after the treatment, and lack of residual effect [14]. Table 2 shows selected studies on *E. coli* inactivation using ultrasonic disinfection.

**Table 2.** Overview of selected studies on ultrasonic inactivation of *Escherichia coli*

Disinfection efficiency: poor =<90% bacteria inactivated, intermediate=90-99.9%, good=99.9%

| US reactor | Frequency (kHz) | Intensity | Suspension type and volume | Time (min) | Disinfection efficiency | Reference |
|---|---|---|---|---|---|---|
| | | | 300 mL | | | [45] |
| **Probe** | 20 | 4.60 $Wcm^{-2}$ | Lab-grade water | ≤60 | Good | |
| **Probe** | 20 | 18.5 $Wcm^{-2}$ | Lab-grade water | ≤60 | Intermediate | |
| **Probe** | 20 | 74.0 $Wcm^{-2}$ | Lab-grade water | ≤60 | Good | |
| | | | 50 mL | | | [46] |
| **Probe** | 20 | 15 $Wcm^{-2}$ | Saline solution | 15 | Poor | |
| **Probe** | 20 | 15 $Wcm^{-2}$ | Stream water | 15 | Poor | |
| **Bath** | 38 | 0.6 $Wcm^{-2}$ | Stream water | 20 | Poor | |
| **Probe** | 27.5 | 42 W/mL | 3 mL, sterile water | 3 | Good | [47] |
| **Bath** | 42 | 70 W | 600 mL, water | 15 | Poor | [48] |
| | | | | 90 | Intermediate | |

**Table 3.** Comparison of different disinfection methods [1,4,7,16,22,49,50]

| Disinfection method | Advantages | Disadvantages |
|---|---|---|
| **Chlorination** | - Extreme effectiveness for bacteria and viruses inactivation<br>- Residual protection<br>- Relatively simple equipment<br>- Well understood method<br>- Established dosing technology | - Formation of DBPs<br>- Ineffective for protozoa and cysts<br>- Possible taste and odour problems<br>- Less effective for turbid and organic-rich waters<br>- pH has big influence<br>- Corrosive chlorine gas<br>- Dependence on chemical access and trained operators |
| **Chloramination** | - Less DBPs formation<br>- Residual protection<br>- Less taste and odour problems | - Need for on-site generation<br>- Lower disinfection efficiency<br>- Dependence on chemical access and trained operators<br>- Potential aquatic toxic effects of chloramine |
| **Chlorine dioxide** | - More resilient to pH changes<br>- Long-lasting residual protection<br>- Effective against Giardia | - Need for on-site generation<br>- More expensive than chlorine<br>- Formation of chlorates and chlorites<br>- Dependence on chemical access and trained operators<br>- Unsatisfactory taste and odours |
| **Ozonation** | - No dependence on chemical access<br>- Most effective chemical method against protozoa and cysts | - Need for on-site production<br>- No residual protection<br>- Higher costs than for other chemical disinfectants<br>- Possible formation of bromates<br>- Energy intensive<br>- Complex equipment<br>- Highly skilled maintenance needed |
| **UV lamps** | - Effective against viruses, spores, cysts<br>- No taste and odour issues<br>- No by-products<br>- No dependence on chemical access | - Low energy efficiency<br>- Regular replacement of lamps<br>- Lamps contain mercury<br>- Waste disposal issues<br>- No residual protection<br>- Efficiency can decrease in case of fouling, biofilm formation, high water, inadequate transmittance of water |

| | | |
|---|---|---|
| **Electrochemical disinfection** | - Direct effect, no need for chemicals<br>- Can have residual effect<br>- Cost effective<br>- Environmentally friendly | - Continuous energy supply needed<br>- Electrodes lifetime<br>- Efficiency affected by deposits on electrodes |
| **Utrasonic disinfection** | - No byproducts<br>- No need for chemicals | - Expensive compared to chemical disinfectants<br>- Continuous energy supply needed<br>- Studies on larger scale needed<br>- No residual effect |

## 3. CASE STUDY

3.*1. Experimental set-up*

Two ultrasonic devices were used in this study: a 20 kHz sonotrode or a horn probe (Fig. 1a) and a dual frequency ultrasonic bath (25/45 kHz) (Fig. 1b). Both devices can operate on different amplitude percentages, meaning that ultrasonic intensities can be varied. Technical data is shown in Table 4.

**Table 4.** Technical data for ultrasonic devices used in this study

| Type | Manufacturer | Model | Frequency (kHz) | System power (W) | Nominal ultrasound power (W) | Delivered power (W) |
|---|---|---|---|---|---|---|
| **Probe** | Bandelin | HD 2200.2 | 20 ± 500Hz | 200 | 200 (4 x 50 W transducers) | 88 |
| **Bath** | Elma Ultrasonics | TI-H 10 MF2 | 25/45 | 800 | 200 | 86/70 |

Nominal ultrasound power usually differs from power transmitted to water (delivered power). The delivered ultrasound power was calculated using the calorimetric method [40]:

$$P = mC_p(\frac{dT}{dt})$$

where *Cp* (4,18 J(gK)) is the specific heat capacity of water and *m* is the mass of sonicated water, *dT* is the temperature increase for the applied sonication time *dt*. One litre of distilled water was treated for 120 seconds, and the temperature increases were recorded. Results of this calculation for 100% amplitude setting is, also, shown in table 4.

According to the paper [51] the conversion rate of the nominal electrical power of the ultrasonic system to the water in insulated vessel is 0.51, i.e. that 51% of the nominal ultrasonic power is transferred to the water. For used ultrasonic bath, this coincides with the calorimetric method. Since here insulated vessel during calorimetry was not used, theoretical value (102 W) of the water delivered power is reduced to 86 W and 70 W, due to the heating of the vessel itself and the heat loss to the surrounding space. To make sure that ultrasonic intensities stayed constant during the experiments, measurements obtained with Cavimeter by NMC were used.

Experiments with a horn probe are performed by submerging the probe into the water filled beaker so that the tip of the probe is 2 centimetres under the water surface. Experiments in a bath were done by

directly pouring the water into the bath. To avoid contamination, all equipment and used vessels were disinfected with 70% ethanol and thoroughly cleaned before and after every experiment.

Experiments investigated the effects of applied ultrasonic device, frequency, sample volume, treatment time and intensity/amplitude on disinfection efficiency of ultrasound. Three volumes of 200, 600 and 1000 mL were tested for 2, 6, 10 and 20 minutes. Before and after every experiment, a sample was taken for microbiological analysis. Also, water quality was tested using Hanna Instruments HI-9829 multiparameter (Fig.1c) with sensors for temperature, pH, oxidation-reduction potential, dissolved oxygen, electroconductivity and turbidity.

*3.2. Water samples and microbial detection system*

Since naturally occurring microorganisms in the environment may show different resistance than cultures grown in the laboratory, we chose to use raw water for our study. Water taken from a nearby lake was used for all experiments. This resulted in varying initial bacterial counts depending on factors such as the time of sample collection and weather conditions. To facilitate comparison between experiments, the number of bacteria was normalised to percentages. Although various bacteria were present in the sample, we focused only on total coliforms and *Escherichia coli* as indicators of faecal contamination of drinking water. A sample was collected for microbiological analysis before and after each experiment. Cell densities or number of bacteria were expressed as number of colony forming units per 100 millilitres of water sample (CFU/100 ml).

TECTA™ B4, an automated fluorometry-based microbial detection system from Pathogen Detection Systems, was used for identification and quantification of *E. coli* and total coliforms. This is a directed enzyme-substrate method combined with a patented Polymer Partition technology that isolates the optical detection path from the water sample. In other words, this system measures the enzyme activity of the targeted bacteria using an optical fluorescence sensor [52]. The system is shown in Fig 1d. Based on the targeted microorganisms and their enzyme preferences, four types of tests are available: Broad spectrum *E. coli* (glucuronidase enzyme), Total Coliforms (galactosidase enzyme), Fecal Coliforms (galactosidase enzyme) and *Enterococcus* (glucosidase enzyme). 100 ml of water sample is poured into the corresponding cartridge containing pre-packaged substrate, the cartridge is tightly sealed and when all the substrate is dissolved, it is placed in the Tecta. Depending on the type of test, samples are incubated at 35, 41.5 or 44.5 °C and the results are reported in maximum of 24 hours. For *E. coli* and total coliforms, the incubation temperature is 35°C, and maximum analysis time is 18 hours. The dynamic range of the system is <1 to $>10^8$ CFU in 100 mL, without the requirement for sample dilution. Full automation of the test analysis and interpretation processes eliminates the need for subjective, visual interpretation of results. Furthermore, operation is simple enough that it does not require specialized personnel. Biggest advantage of this system is time-saving; for detection of samples with 100 CFU/100 ml the analysis is finished in about 9 hours, and for samples with $10^6$ CFU/100 ml in just four hours.

a)  b)  c)  d) 

**Fig. 1.** a) Horn probe set-up, b) ultrasonic bath set-up with cavimeter, c) multimeter, d) TECTA B4

*3.3. Results and discussion*

Experiments were carried out with volumes of 200, 600 and 1000 mL of raw water sample by using the ultrasonic probe operating at 20 kHz and the ultrasonic bath operating at both 25 and 45 kHz. The water was treated for 2, 6, 10, 20 and in some cases 30 minutes. Since the intensity on both ultrasonic devices, can be controlled via amplitude percentage, experiments were performed at 20, 60 and 100%. In all experiments, an initial rise in number of colony forming units of *E.coli* and total coliforms was observed. However, this rise was more pronounced when lower intensities were used. The disinfection efficiencies of the systems were determined as log kills (log reduction) following the expression:

$$\log \text{kill} = \log(\frac{N_0}{N})$$

$N_0$ is an initial number of colonies per 100 mL of sample and N is the number of the colonies per 100 mL sample after the treatment. Log reduction or log kill indicates a 10-fold reduction in the number of microorganisms, meaning that with every step their number is reduced by 90%. So, 1-log kill indicates 90% removal of existing bacteria, 2-log kill means 99% removal, 3-log kill achieves 99,9% of disinfection effect and so on. At low intensities and treatment time less than 6 minutes, especially, for volume of 1000 mL the efficiency of ultrasonic disinfection is poor and the "de-clumping" effect is observed, as discussed in section 3.3.1. The results of the experiments conducted at 100% amplitude setting are summarised in the Table 5, where EC stands for *Escherichia coli* and TC for total coliforms. In experiments performed with 200 mL of the sample, complete inactivation of both *E. coli* and total coliforms was achieved in 10 minutes of treatment time in a horn probe set-up, while more time was required in a bath. When 600 mL of the sample was used, 30 minutes of horn probe treatment was needed for complete inactivation of the bacteria present and the highest log kill was achieved in a bath operating at 45 kHz for 20 minutes. In 20 minutes of treatment time of 1000 mL of the sample in both set-ups resulted in less than 1-log reduction. If the surface area of the homogenizer probe (TT 13 probe with the surface area of 132.73 $cm^2$) is taken into account, the effective ultrasonic intensity of the homogenizer is 0.663 $W/cm^2$. Accordingly, for the total surface area of the four ultrasonic bath transducers (1590 $cm^2$ per transducer), the resulting effective ultrasonic intensities are 0.215 $W/cm^2$ for 25 kHz and 0.175 $W/cm^2$ for 45 kHz. These intensities also confirm the results. The homogenizer, which has a higher intensity, will for the same volume (200 mL), remove bacteria sooner than the bath. As the bath has a lower intensity, it takes 10 min more for 100 % efficiency, while for 600 mL only 99% efficiency is achieved in 20 min. The comparison of efficiency for these experiments is shown in Fig. 3-5. Both indicators, *Escherichia coli* and total coliforms exhibited similar behaviour, as shown in Fig. 2. Regarding the effect of ultrasonic treatment on the quality of the water, a significant increase in temperature and turbidity is evident. Temperature rise is expected due to the partial conversion of transmitted acoustic energy in the medium into heath. Increase in water turbidity after ultrasonic treatment could be caused by long delays contact times, high density, and transducers wear [14].

**Table 5.** Results overview for experiments conducted on 100% amplitude setting

| | | | | **CFU reduction, %** | | **Log kill** | |
|---|---|---|---|---|---|---|---|
| **Volume** | Set-up | Frequency | Time, min | EC | TC | EC | TC |
| **200 mL** | probe | 20 kHz | 6 | 99.954 | 99.951 | 3.33 | 3.32 |
| | | | 10 | 100 | 100 | >6 | >6 |
| | bath | 25 kHz | 6 | 28.408 | 14.825 | 0.15 | 0.07 |
| | | | 10 | 99.961 | 99.981 | 3.41 | 3.71 |
| | | | 20 | 100 | 100 | >6 | >6 |
| | | 45 kHz | 6 | 80.859 | 56.646 | 0.72 | 0.36 |
| | | | 10 | 97.594 | 99.752 | 1.62 | 2.61 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 20 | 100 | 100 | >6 | >6 |
| **600 mL** | probe | 20 kHz | 20 | 99.997 | 99.994 | 4.53 | 4.23 |
| | | | 30 | 100 | 100 | >6 | >6 |
| | bath | 25 kHz | 10 | 93.478 | 95.104 | 1.19 | 1.31 |
| | | | 20 | 99.220 | 99.939 | 2.11 | 3.21 |
| | | 45 kHz | 10 | 51.173 | 55.669 | 0.31 | 3.18 |
| | | | 20 | 99.934 | 99.942 | 0.35 | 3.24 |
| **1000 mL** | probe | 20 kHz | 20 | 45.151 | 28.142 | 0.26 | 0.14 |
| | bath | 25 kHz | 10 | 94.277 | 89.183 | 1.24 | 0.97 |
| | | | 20 | 99.841 | 99.758 | 2.80 | 2.62 |
| | | 45 kHz | 10 | 51.589 | 44.478 | 0.32 | 0.26 |
| | | | 20 | 65.874 | 39.096 | 0.47 | 0.22 |

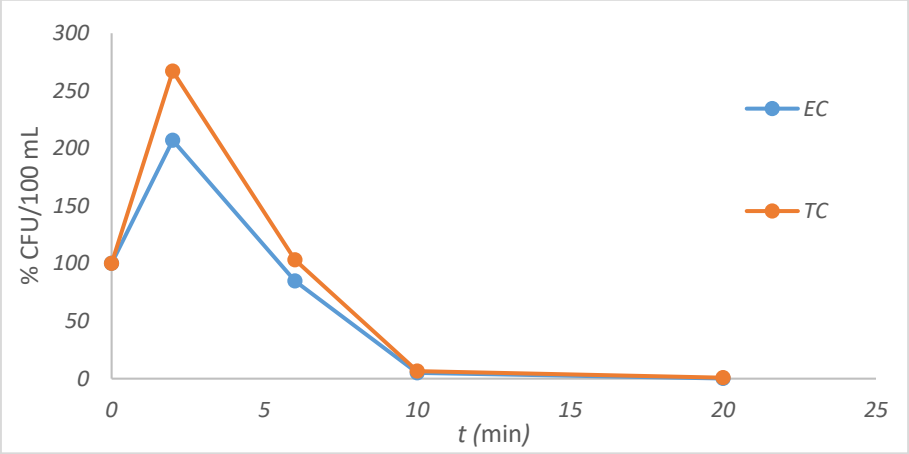


**Fig. 2.** Change in number of colonies in an experiment with 600 mL of the sample being treated in a bath at 25 kHz

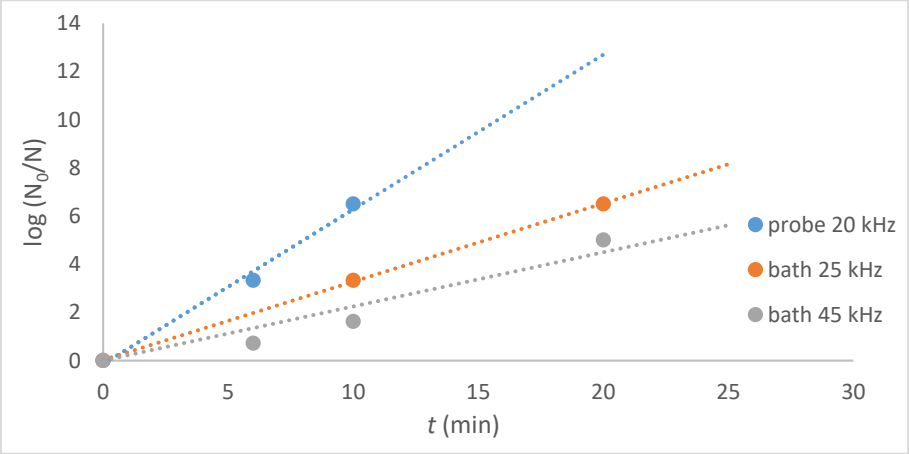


**Fig 3.** Efficiency of *E.coli* inactivation in experiments with 200 mL of the sample water

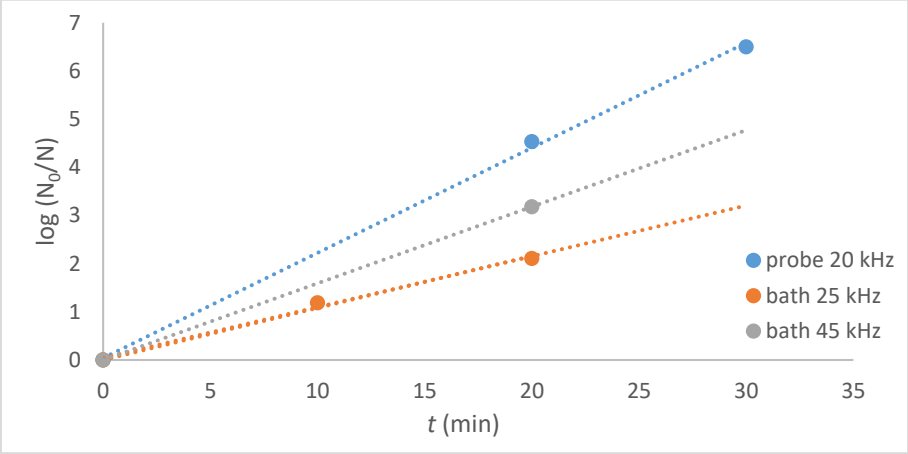


**Fig. 4.** Efficiency of *E.coli* inactivation in experiments with 600 mL of the sample water

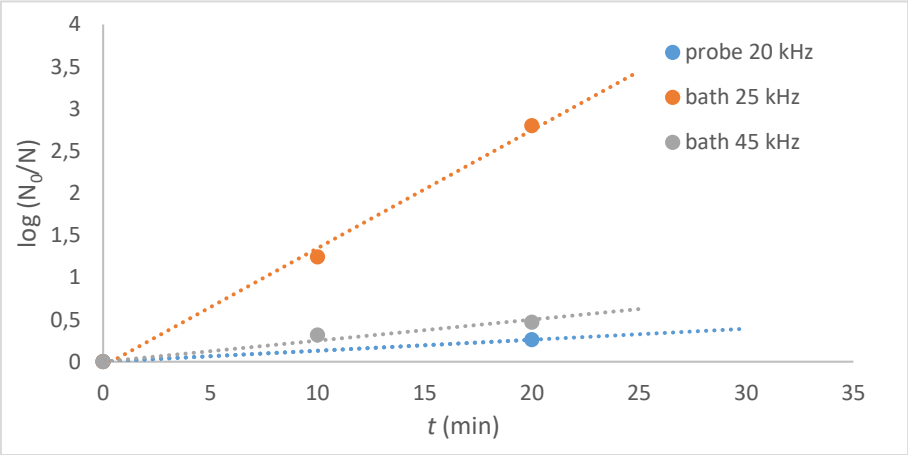


**Fig. 5.** Efficiency of *E.coli* inactivation in experiments with 600 mL of the sample water

*3.3.1. "De-clumping effect"*

Low intensity ultrasound has a significant effect on particle size distribution and can eliminate large particles [53]. This effect for bacteria can be observed as "de-clumping" effect, where bacterial flocs deagglomerate under the influence of mechanical shear stress [53]. The efficiency of the disinfection technique often depends on the suspended solids concentration [54,55], as suspended particles can shield bacteria from the disinfectant [56]. Furthermore, if a cluster of bacterial cells is formed, the disinfectant cannot properly react with the cells within the cluster. For example, the efficiency of ultraviolet irradiation is diminished in the presence of high concentrations of suspended solids, due to UV light scattering, shading of bacteria and incomplete penetration into "sheltering" flocs. The disintegration of bacterial flocs makes ultrasonic treatment great addition or pre-treatment to other disinfection techniques such as UV, chlorine, or ozone treatments. The overall effect of applied ultrasound is the result of competition between two main occurrences caused by acoustic cavitation effects. These are bacterial de-clumping (breaking up agglomerates into more individual colonies in water) and bacterial inactivation. An initial rise in the number of bacteria, observed in nearly all of the experiments can be attributed to bacterial splitting. In experiments with 200 mL of sample, bacterial killing started after 2 minutes and in experiments with 600 mL after 6 minutes. When 1000 mL of sample was used, de-clumping effect was dominant for about 10 minutes of treatment time. The change in the number of *E. coli* colonies during the experiments depending on the volume of treated water is shown in Fig. 6-8 for each device used at 100% amplitude setting. It can be concluded that irradiation time, intensity and reactor configuration have a strong influence on efficiency of ultrasonic disinfection [14]. For significant microbial inactivation, higher intensities and/or exposure time are needed.

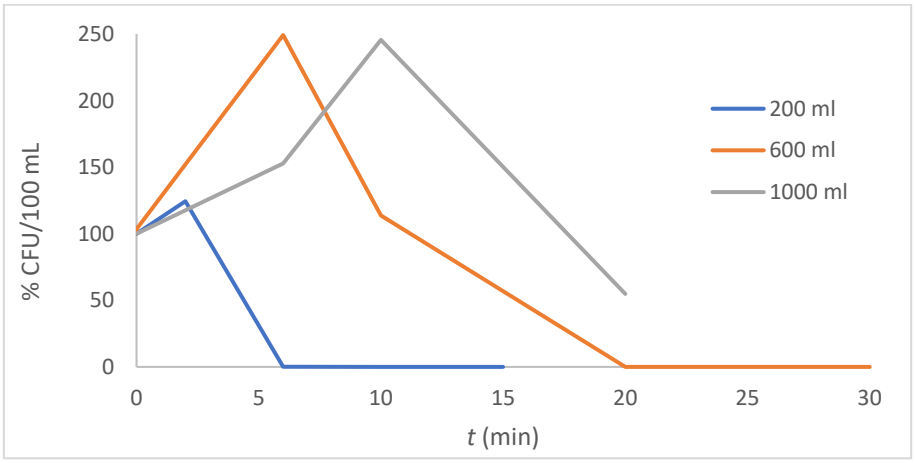


**Fig. 6.** Change in the number of *E. coli* colcolonies during the experiments with the horn probe

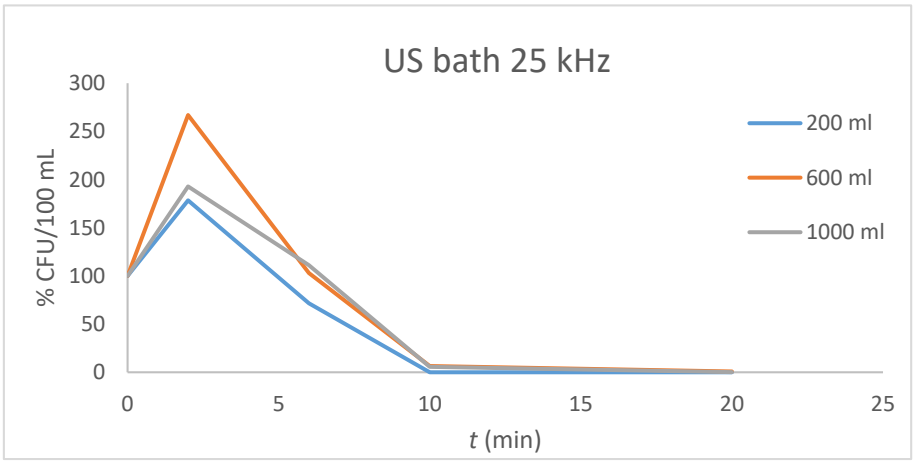


**Fig. 7.** Change in the number of *E. coli* colonies during the experiments in the US bath at 25 kHz

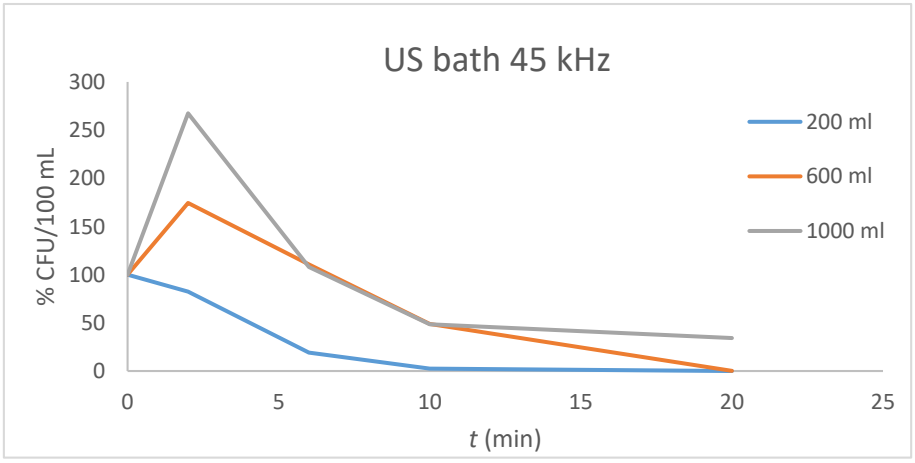


**Fig. 8.** Change in the number of *E. coli* colonies during the experiments in the US bath at 25 kHz

*3.3.2. Temperature effect*

As mentioned before, ultrasonic treatment results in significant temperature rise if no cooling is provided, especially for smaller volumes. In order to examine the effect of heating on overall ultrasonic disinfection efficacy, a series of experiments with cooling was conducted. Ice jacket was used to keep

the temperature at 20 ± 1°C in the probe reactor, and at 24±1 °C in the bath reactor. Effect of the cooling on the disinfection efficiency is shown in Fig. 8a for experiment with 600 mL and the probe and in Fig. 8b for experiment with 1000 mL and the bath. For experiments that resulted in more than 99% bacteria inactivation when no cooling was provided, pure ultrasonic effect resulted in only 77% (600 mL, probe) and 37 % (1000 mL, bath) bacterial inactivation. It can be concluded that the temperature rise is a significant factor in ultrasonic disinfection and that the effect of ultrasonically produced heath is greater than the mechanical effects of ultrasound, especially on larger volumes.

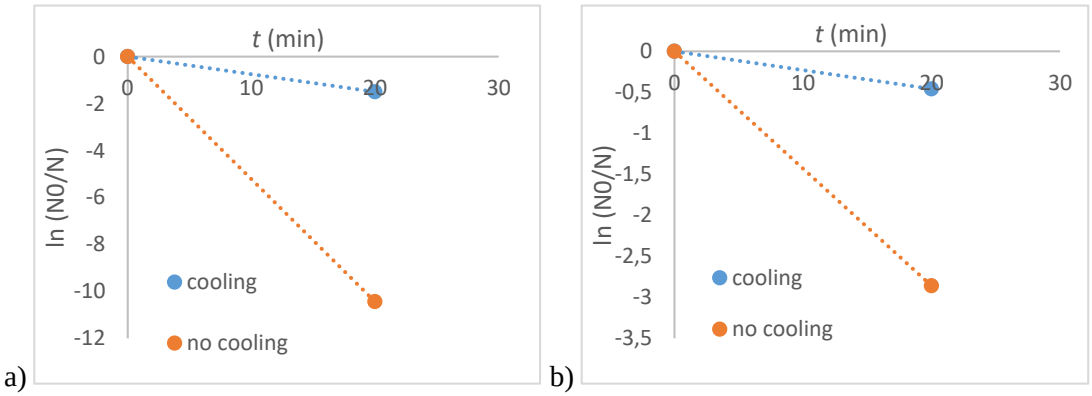


**Fig. 8.** Disinfection efficiency in time for experiments with cooling and without: a) 600 mL and probe b) 1000 mL in the bath

## 4. CONCLUSIONS

Disinfection of potable water plays a critical role in the protection of public health. Various disinfection methods have been proved to be very effective, but they all have certain drawbacks. The adequacy of chosen technology can be decided based on disinfection efficiency, residual activity, disinfection by-products formation, treated water quality, applicability and operational and initial costs.

Ultrasonic technology can be successfully applied for inactivation of E. coli and total coliforms in drinking water. However, high intensity and long exposure times are needed for total inactivation, meaning that this technology in nowadays available systems is not economically feasible as a stand-alone technology. Due to the existing de-clumping effect some prolonged treatment time should be taken into account before bacterial inactivation takes place. This leaves questionable the possibility of using conventional ultrasonic systems for the treatment of large water quantities in short time, thus continuous ultrasonic treatment systems. However, according to the results obtained with the horn probe for 200 mL and 600 mL, after bacterial inactivation takes place, larger volumes require a longer treatment time where a linear relationship is observed. Three times larger volume thus required three times longer treatment time for full bacterial inactivation. Also, ultrasonic treatment does not provide residual protection against possible recontamination. Costs and efficiency can be optimized by combining this technology with methods such as heating (usually inherent to ultrasonic systems), UV irradiation, ozonation or chlorination. Due to ultrasounds' ability to break up bacterial flocs, it is a valid addition to conventional water treatment systems.

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