Disinfection of *Cryptosporidium parvum* Oocysts in Water Using Ultrasonic Treatment

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ABSTRACT

Ultrasound in a liquid phase cause mass and heat transfer across the liquid through cavitational processes which act as nanoreactors to generate unstable mechanical equilibrium. The effect of 1 MHz ultrasound on the inactivation of *Cryptosporidium parvum* was investigated. Continuous irradiation of ultrasound (20 min) increased temperature due to cavitational phenomena. Ultrasound irradiation of liquid containing *Cryptosporidium parvum* showed significant quantitative changes in pH, temperature and inactivation of *Cryptosporidium parvum* (102.7 oocysts killed/s) with a minimum energy consumption (0.05 oocysts/s).

Keywords: cavitation, *Cryptosporidium parvum,* inactivation, ultrasound, viability,

1 INTRODUCTION

In recent years it has been confirmed that ultrasound is capable of producing cell lysis and other deleterious effects, such as membrane impairment, DNA degradation, organelle damage, and miscellaneous functional and biochemical changes. Ultrasound application in water treatment has been noted as an effective technique for disinfection [1]. During irradiation of ultrasound in aquatic solutions the ultrasound wave and free radicals such as OH^-, HO_2^- and O^- are induced by violent collapse of cavitation bubbles [2]. During disinfection processes using ultrasound wave generates free radicals transfer into the bulk solution to disintegrate cellular membranes (first stage in disinfection) and further recombination of free radicals to form as an oxidant (the second stage of the disinfection). During the cavitational collapse, bacterial biomass is susceptible to inactivation due to penetration of free radicals and act as a biocide.

Viral and protozoan pathogens such as *Cryptosporidium parvum* are resistant to chlorination disinfection [3,4]. *Cryptosporidium parvum* is a protozoan parasite which is a highly infectious organism. In 1993, *C. parvum* was responsible for over 300,000 infections in Milwaukee, Wisconsin when oocysts (the infectious stage of the microorganism) escaped treatment barriers and entered the drinking water system. Since ultrasound technology has potential applications in water and wastewater treatment, we were interested in evaluating its ability to inactivate *Cryptosporidium parvum* oocysts. The specific objective of this study was to determine whether 1 MHz ultrasound technology can influence the viability of *C. parvum* oocysts.

2 MATERIALS AND METHODS

The experimental ultrasound (1 MHz frequency) apparatus was designed in the Electrical Engineering Department at Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM), Mexico City. The unit had a specific pulsed frequency of 1 MHz, 4.1 W of power output, with an intensity of 2.3 W/cm^2. A sample solution of 100 mL was irradiated in a beaker where the ultrasound was transmitted into the solution through a piezo-electric transducer (Fig. 1). The emitted frequency and out-put power was analyzed using Ultrasound Power Meter (UHMIC Instruments, USA).

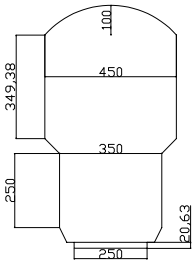

Figure 1: Piezo-electric transducer used for ultrasonic sonication treatment (scale 10 units = 1mm).

Laboratory studies using the 1 MHz unit were carried out in 100 mL beakers containing 60 mL of KI (0.06 M) samples [5]. Twenty one samples were prepared. The samples were irradiated in triplicate for 2, 4 and 10 minutes, under atmospheric conditions. The pH and temperature of the samples before and during ultrasonic treatment were recorded at “one” minute intervals. Viable *Cryptosporidium parvum* oocysts were obtained from a commercial source (Waterborne, Inc, New Orleans, LA) and laboratory studies were performed at the laboratory of Food Science and Microbiology in Texas A&M University, College Station, Texas. Sixty milliliter samples were inoculated in distilled water with a defined volume of 90%

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viable *C. parvum* oocysts. The oocysts concentration in each sample was approximately 3.3 X 10^2 oocysts/mL. The ultrasonic treatments were performed at room temperature (24-25°C). Triplicate samples were exposed to 1 MHz ultrasonic frequency for 2, 4, and 10 minutes. A control unexposed sample was also included in the study. After the ultrasonic treatment, the sample culture was centrifuged at 2700 rpm for 15 min. The supernatant was carefully removed and the "pellets" were analyzed using the standard USEPA method 1623 [6]. Samples were stained as per IFA protocol [7] with Propidium Iodide (1 mg/mL in 1 X PBS) to determine the viability of oocysts.

3 RESULTS AND DISCUSSION

Treatment of aqueous solutions with ultrasound wave generates rarefactions and compressions. When ultrasound wave generates sufficiently high amplitude, it produces cavitation, considered to be a key parameter that generates physicochemical changes in the liquid phase. When drinking water (without any microbial inoculation) is exposed to 1 MHz ultrasound, a temperature increase is noted along with a distinct pH fluctuation (Fig. 2). The mean (n=3) temperature and pH values are shown in Figure 2.

Temperature increases shown in Figure 2 can be attributed to discharge waves generated from the cavitational zone of the liquid phase. The discharge waves create high temperature (more than 5500°C) and pressure (50,000 kPa) within a short period of time (nanoseconds) in the cavitaional zone of the liquid phase [8]. This phenomenon is presented in Figure 2 as gradient temperature in the liquid phase (water treated with ultrasound). The pH fluctuations can be attributed to bubble cavitation [9]. During cavitation, the bubbles acting like nano-reactors in which the cavity acts as a source of H^+, OH^- and HOO^- radicals, resulting in pH fluctuations (Fig. 2). The phenomenon developed is shown in equations (1) to (6). These results suggest that the change in temperature and pH could be exploited as control parameters during 1 MHz ultrasound treatment process.

$$H_2O \rightarrow H^+ + OH^- \quad (1)$$

$$O_2 \rightarrow 2O^- \quad (2)$$

$$H^+ + O_2 \rightarrow HOO^- \quad (3)$$

$$O + H_2O \rightarrow 2OH^- \quad (4)$$

$$2HOO^- \rightarrow H_2O_2 + O_2 \quad (5)$$

$$2OH^- \rightarrow H_2O_2 \quad (6)$$

The influence of 1 MHz ultrasound irradiation on the viability of *C. parvum* oocysts is shown in Fig. 3. Viability was calculated as a % of viable oocysts as compared to non-viable oocysts. The stock *C. parvum* sample obtained from the commercial source contained 90% viable oocysts. Within 2 minutes of exposure to 1 MHz ultrasound viability decreased by 87.82%. The number of non-viable oocysts increased to 94.02% by 4 minutes. Finally, the number of non-viable oocysts increased to 94.02% after 10 minutes of exposure. Fig. 3 shows the main effect plot for the *C. parvum* destruction. Results presented in Fig. 3 indicate that 2 min of treatment is not the optimum period of treatment time whereas, 4 min is considered to be optimum for energy safe with significant destruction of *C. parvum* oocysts. The non-viability as determined by this assay is based on the apparent permeability of the oocysts membrane to the dye Propidium Iodide (1 mg/ml in 1 X PBS).We hypothesize that the cavitation during the 1 MHz ultrasonic irradiation caused damage to the membrane [10].

A statistical analysis was performed to determine the significance of the difference between 2, 4 and 10 min. of treatment time. Analysis of Variance (ANOVA using Minitab 14) for *C. parvum* destruction after 2 and 4 min treatments, showed significant difference level at 4 min of treatment time ($p = 0.048$). Similar ANOVA was performed to identify the statistical difference between 4 and 10 min of treatment time. From the results of the statistical analysis it is concluded that 10 minutes of treatment time was not statistically significantly different ($p = 0.941$) than 4 minutes of treatment time.

The disinfection rate (conversion of viable oocysts to non-viable oocysts) was calculated based on the best fit of the equation published by Jyoty and Pandit [11]. Mathematically, it is expressed in equation (7).

$$\frac{dc}{dt} = k(C)^n \quad (7)$$

where dc/dt is the rate of disinfection [(viable oocysts/mL)killed/time]; k is the disinfection rate constant (s^{-1}), C the viable oocysts concentration (oocysts/mL) and n the exponential of the oocysts concentration at any irradiation time considered as first order. The overall rate of disinfection (non-viable oocysts/unit time) is equal to (d*c*/d*t*)*(Volume treated oocysts/mL) oocysts/mL killed/time (mL). The disinfection rate was calculated (8-12).

$$\frac{dc}{dt} = kC \quad (8)$$

$$\int_{C_1}^{C_2} \frac{dc}{C} = k\int_0^t dt \quad (9)$$

$$\ln\frac{C_1}{C_2} = kt \quad (10)$$

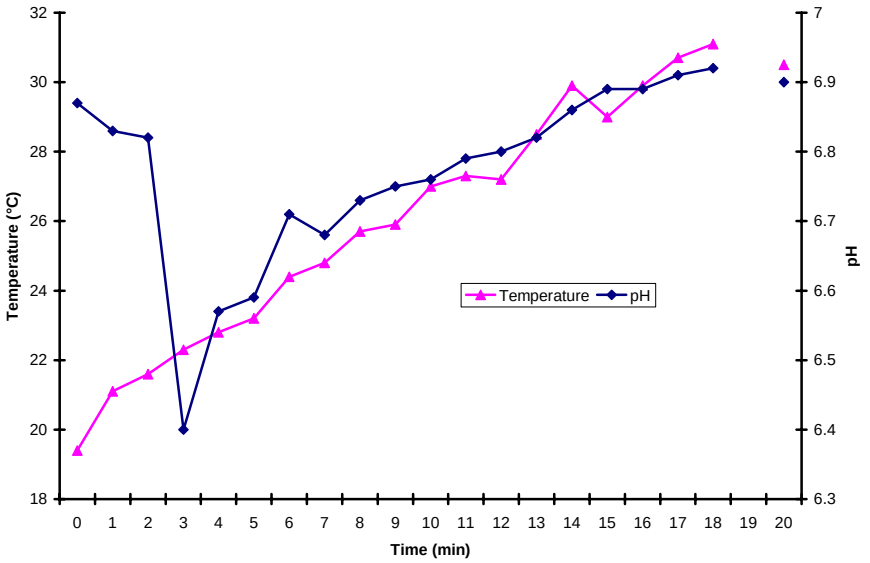

Figure 2: Temperature and pH variation during 1 MHz ultrasound sonication.

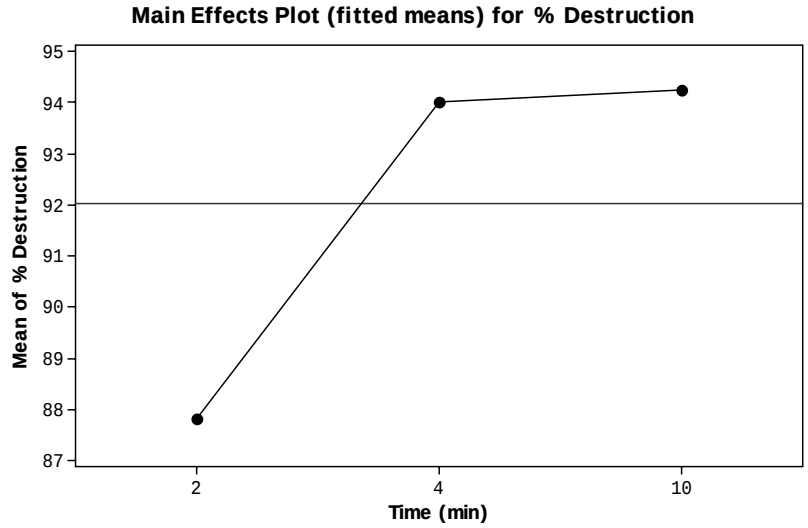

Figure 3: Effect of 1 MHz ultrasound irradiation on viability of *Cryptosporidium parvum* oocysts.

$$k = \frac{\ln \frac{C_1}{C_2}}{t} \quad \text{(Disinfection rate constant)} \qquad (11)$$

The overall rate of disinfection is:

$$non-viable oocyts/s = k \times C \times V \qquad (12)$$

Based on Table 1, it is evident that 10 minutes of 1 MHz provides the maximum level of non-viable oocysts. However, the most significant increase in the numbers of non-viable oocysts was observed after 2 minutes of ultrasonic irradiation (Fig. 3). There was a difference of 70 non-viable oocysts between 10 minutes and 2 minutes of treatment. The overall rate of disinfection (ie., number of non-viable oocysts per second) is approximately 102, 75, and 33 oocysts respectively (Table 1). The corresponding energy and monetary costs to achieve this disinfection rate is shown in Table 2 and Table 3. Irrespective of whether the sample was exposed for 2 minutes or 10 minutes, the number of oocysts made non-viable in 100 mL of sample is approximately 7 oocysts/W of power and less than 0.1 oocysts/J of consumed power (Table 2). The cost (per Mexican rates) of operating a 1 MHz ultrasonic unit for 2 minutes and 10 minutes correspond to approximately US $0.86 and US $4.13 per m^3 of water. Based on these costs,

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Treatment time (minutes)	2	4	10
Initial concentration (Viable oocysts/mL)	121	121	121
Final concentration (Viable oocysts/mL)	4.667	0.889	0.533
Disinfection rate constant k (s^{-1})	0.027 s^{-1}	0.020 s^{-1}	0.009 s^{-1}
Average concentration (Viable oocysts/mL)	62.833	60.944	60.767
Volume treated (mL)	60	60	60
Overall rate of disinfection (oocysts killed/s)	102.272	74.864	32.962

Table 1: Ultrasound (1 MHz) sonication on overall rato of disinfection of *Cryptosporidium parvum*.

Treatment time (minutes)	2	4	10
Volume treated (mL)	60	60	60
Electrical consumption (W)	17.6	17.6	17.6
Initial concentration (Viable oocysts/mL)	121	121	121
Final concentration (Viable oocysts/mL)	4.667	0.889	0.533
Total oocysts killed/mL	116.333	120.111	120.467
Oocysts killed/W	6.610	6.824	6.845
Total oocysts killed/J of power consumed	0.055	0.028	0.011

Table 2: Energy efficiency and cost of treatment using ultrasound 1 MHz sonication for disinfection of *Cryptosporidium parvum*.

Treatment time (minutes)	2	4	10
Energy efficiency (Oocysts killed/J)	0.055	0.028	0.011
Energy required to eliminate total oocysts (J/100mL)	1815.473	3516.744	8765.91
In kWh/L	0.0084	0.0163	0.0406
Cost in Mexico per kWh*	$0.102	$0.102	$0.102
Cost of treatment ($/$m^3$)*	0.856	1.658	4.134

*Cost in US dollars

Table 3: Effect of ultrasound sonication on *Cryptosporidium parvum* disinfection and energy efficiency.

it would appear that a 2 minutes exposure would be more cost effective. However, it should be noted that the number of surviving oocysts after 2 minutes and 10 minutes of ultrasonic treatment correspond to 4.66 oocysts/mL after 2 minutes and less than 1 oocysts/mL after 10 minutes. Viability does not correspond to infectivity since viable oocysts are not necessarily infectious. However, knowing the highly infectious nature of *C. parvum* oocysts it can be argued that having 5 viable oocysts per milliliter of water can be very expensive for human health perspective.

4 CONCLUSIONS

This pilot laboratory study has demonstrated that 1 MHz ultrasonic irradiation of water to inactivate *C. parvum* oocysts is technically and economically feasible. Ultrasound sonication is cost efficient technology to disinfect *Cryptosporidium parvum.* In laboratory scale it showed effectiveness in disinfection with significant cell lysis.

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