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DISINFECTION EFFICIENCY FOR *ESCHERICHIA COLI* INACTIVATION IN WATER USING LOW FREQUENCY ULTRASONICATION: EFFECT OF TEMPERATURE, POWER, AND VOLUME CHANGE ON INACTIVATION

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ABSTRACT

The present study explored the application of 20-kHz ultrasonic processor for water disinfection in conjunction with the thermal effect of the process. The experiments were divided into three stages: variable temperature, variable amplitude, and variable volume. Temperature affects the ultrasonic treatment significantly by reducing the treatment time. For controlled temperature experiment, 5 log (base 10) reduction was achieved after 30 minutes of treatment, as compared to the 10 minutes of contact time for experiment without temperature control. The disinfection efficiency of E. coli increased with increase in the power input, through change in amplitude, from 0.140 log kill/min at the lowest power (122 W/L) to 0.799 log kill/min at the highest power (310 W/L). Power is directly proportional to temperature; wherein, maximum temperature of 61°C was attained utilizing power input of 310 W/L. As for the effective volume of the sample, increasing the volume reduces the concentration of the energy being dissipated in the sample. After 30 minutes of treatment the maximum percent inactivation for 500 mL and 1000 mL are 98.30% and 97.35% respectively. Overall, there exists a synergy between ultrasound and heat, such that ultrasonic treatment increased the vulnerability of E. coli to heat.

1. INTRODUCTION

The presence of pathogenic microorganisms in water is a worldwide public health problem. It was estimated by the World Health Organization (WHO) that water-related disease caused the death of 3.4 million people, mostly children [1]. Therefore, the need to utilize effective water disinfection for the prevention of these diseases, and most importantly pathogenic microbial inactivation, is very crucial.

An array of chemical and physical disinfection techniques is available, ranging from chlorination, ozonation, microwave radiation, and ultraviolet light [2]. Chemical techniques are commonly used, but on the contrary, they produce toxic, mutagenic and/or carcinogenic by-products [3,4]. One example is the trihalomethanes (THMs), a carcinogenic by-product of chlorination [5]. In addition, the effectiveness of physical techniques on microbial inactivation is limited. For ultraviolet light, it is limited when organisms are competent of photoreactivation [6]. The drawbacks of all these disinfection techniques outweigh their efficacy; thus, the necessity for safe, effective and new technology is in order to supplant conventional destructive techniques [4].

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For the actual experiment, five beakers – each containing 100 mL of the seeded sample, were exposed to sonication at various durations (2 min, 4 min, 6 min, 8 min, and 10 minutes). The frequency and amplitude delivered were fixed at 20 kHz and 100 %, respectively. Temperature was allowed to rise and recorded with a thermocouple for every increment of one minute.

To determine the effect of temperature, stage 1 of the experiment involve two sets of experiments. The difference between the two sets was on the use of a flexiglass box. Set 1 was without the use of the box. For both sets, prior to and subsequent to sonication, pH reading was carried out and microbial enumeration was conducted to determine the initial and final count of the microorganism. Four replicates were conducted for this stage.

Heat treatment

In order to determine the contribution of heat to the inactivation of the *E. coli*, a test using heat alone was conducted. This involved heating a 100 mL seeded sample with hot plate for 2 min, 4 min, 6 min. 8 min and 10 minutes. The temperature change was recorded using a thermocouple. Microbial analysis was also conducted before and after the heat treatment.

2.2.2 Stage 2: Sonication at variable amplitude

For this stage, amplitude was varied (100%, 80%, 60% and 40%) to change the delivered power. The set-up was identical to stage one, five beakers – each containing 100 mL of the seeded sample were treated with the ultrasonic processor at 20 kHz for 2 min, 4 min, 6 min, 8 min and 10 minutes.

Before and after sonication, microbial enumeration was conducted to determine the initial and final count of the microorganism. Four replicates were conducted for each change in amplitude. This stage will determine the relationship between time, power generated, and amplitude; thus, determining the ideal amplitude and exposure time for efficient inactivation of the microorganism.

2.2.3 Stage 3: Sonication at variable volume

The last stage of the experiment is important in establishing the efficiency of the low frequency ultrasonication; wherein, efficiency was correlated to the time needed for the inactivation of the *E. coli* at a certain volume. Prior to sonication treatment, microbial enumeration was conducted to determine the initial count of the *E. coli*. Subsequently, 500 mL and 1000 mL of the seeded sample were placed in beakers and subjected to disinfection treatment for 5 min, 10 min, 15 min, 20 min, and 30 minutes.

2.3 Microbial Analysis

The Plate Count Method, a solid-culture medium, was utilized for microbial analysis. Violet red bile agar was used as growth medium. Dilution of the samples before and after treatment was necessary to regulate the count between 30 to 300 colonies per plate. The samples (10 mL) were diluted four to five times using the dilution bottles. About 1 mL of the last two diluted sample was pipeted into each 115 mm petri dishes, starting with the highest dilution, and 5 mL to 10 mL of the liquefied culture media was then added. After mixing and solidifying the media, the petri dishes were inverted and incubated at 37 °C for 24 hours.

2.4 Disinfection efficiency

Disinfection rate or level of inactivation is generally referred to in terms of "log inactivation." It is determined by $-\log(N/N_o)$, where N is the final coliform concentration at a given exposure time, and No is is the initial coliform concentration.

When temperature is incorporated, log inactivation at low power inputs would result to lower correlation coefficients (< 0.95). The reason for this can be attributed to shouldering effect; wherein, inactivation of the bacteria is low in the first few minutes of treatment then significantly increases as

time progress. According to Madge and Jensen (2002), this can be corrected, if by similar analogy the Chick-Watson law can be used for describing the disinfection of water by means of sonication process utilizing power.

3. RESULTS AND DISCUSSION

3.1 Sonication and heat treatment at variable temperature

The ultrasonication process produces enormous amount of energy resulting to the creation of heat. Consequently, during ultrasonic treatment the temperature of the water notably increased as time progressed. Figure 2 shows the typical temperature profile of the water subjected to ultrasonic treatment for 30 minutes at a power input of 310 W/L.

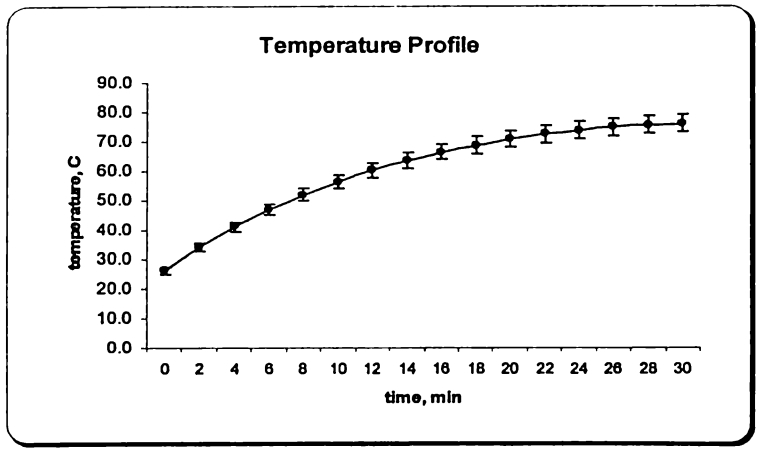

Figure 2. Typical Temperature Profile using at 20 kHz, Sample Volume=100mL, and Power=31W

As evident from the graph, average of three trials, the first few minutes of treatment resulted to an abrupt change in temperature and then the temperature gradually rises. Then, it will come to a point when it tapers off when temperature change is zero. Theoretically, this happens when the system reached an equilibrium, in which the interaction between the medium and the energy produced by the sonicator causes no further change.

3.1.2 Disinfection kinetics

As anticipated, with power input of 310 W/L and allowing temperature to rise, shouldering is evident in this stage of the experiment. Several investigators have cited the evidence of shouldering in thermal inactivation: Mattick et.al. (2001) on heat tolerance of *Salmonella*; and Davey et.al. (2001) on thermal death of bacteria. According to Davey et.al. (2001), shouldering effects are brought about by the clumping separation during heat treatment and the protective effect of dead cells.

A summary of the disinfection data were determined using the disinfection rate constants, k, in which the linear regression of $-\log (N/N_o)$ was plotted against time. Figure 3 shows that in the first few minutes, reduction of *E. coli* was only less than 1-log. The temperature, approximately 39°C after 2 minutes, was not high enough to expect significant kill. Then the log reduction notably increased after 6 minutes of treatment. At this time, there is synergistic effect between heat and ultrasound. The maximum reduction of 5-log units of *E. coli* was achieved after only 10 minutes of ultrasonic treatment. Additionally, as time progressed inactivation increased with increase in temperature.

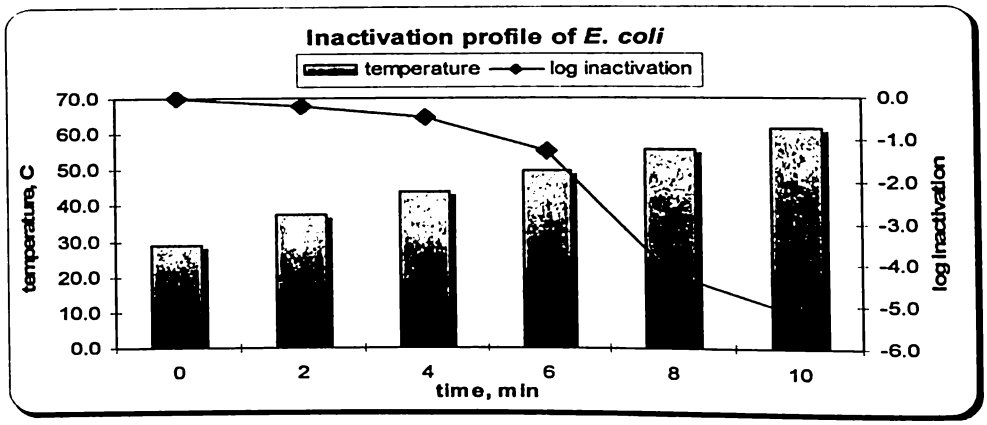

Figure 3. Microbial Inactivation of *E. coli* at 20 kHz, Sample Volume=100mL, and Power=31W

As a result, for power input of 310 W/L, correlation coefficient (r = 0.925) was low because of shouldering effects. In spite of this, the majority of the experiments were collected using different volume of the sample and modifying the amplitude. The log kill at those conditions were more linear with time. Therefore, the Chick-Watson law is appropriate.

Figure 4 is a comparison of the results of this research, ultrasonication with variable temperature (US + heat) and heat alone, and Vo (2006). The ultrasonic processor, microorganism and the media utilized for this study are the same with the one employed by the latter. In this study, 99.999% inactivation (US + heat) was achieved at a shorter time than that of Vo (2006). The time difference was approximately 20 minutes; in which, the large disparity must be ascribed to temperature. The latter conducted the experiments under temperature – controlled conditions, whereas temperature was not controlled in this research. Allowing the temperature to increase also facilitate in the inactivation process as shown in the figure. Given that cavitation is the main contributor to the damaging effect of ultrasonication, any factor that enhances cavitation increases the efficiency of the treatment.

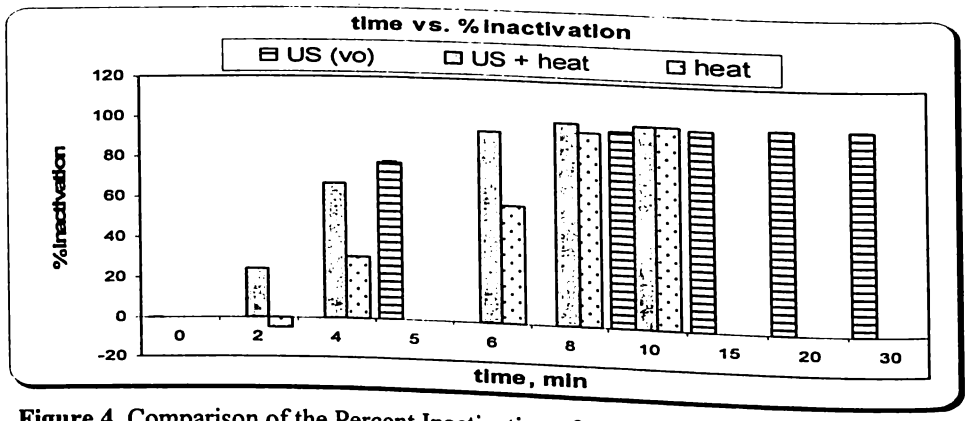

Figure 4. Comparison of the Percent Inactivation of *E. coli* at Controlled and Variable Temperature using 20 kHz, Sample Volume=100mL, and Power=31W

Table 1 indicates that the use of heat alone would ensure inactivation (3 log-reduction) of the *E. coli* after 10 minutes of treatment. Moreover, it illustrates that ultrasonication with heat is ideal for effective disinfection using low frequency ultrasonicator, having attained 5 log-reduction in 10 minutes. The negative value for the percentage inactivation for heat treatment alone is due to shouldering; wherein, within the first two minutes of heat treatment the concentration of the *E. coli* increased.

Table 1
Comparison of the Inactivation Rate

Exposure Time, min	heat		ultrasonication + heat	
	Log(N/No)	%Inactivation	Log(N/No)	%Inactivation
0	0	0	0	0
2	0.02117	-5.0182	-0.18091	33.9700
4	-0.15964	30.742	-0.48027	66.8653
6	-0.38181	58.397	-1.26291	94.3394
8	-1.70725	95.554	-4.20885	99.9937
10	-3.57900	99.973	-5.19632	99.9994

A maximum of 61°C was achieved after 10 minutes of sonication with heat and the treatment with heat alone. Temperature this high (approximately 50°C to 60°C), according to the studies made by Spinks et.al. (2006), Donasco (2000), Sommer et.al. (1997), Salvato (1992), and Russell and Harries (1966), is enough to inactivate coliforms (e.g. *E. coli*). Although in their studies, sufficient contact time was needed for effective disinfection, i.e. within the range of 15 minutes to 120 minutes. But for this study, a few second is enough to achieve effective inactivation using ultrasonication and its byproduct, heat. Thus it illustrates the synergistic effect of ultrasound and temperature in inactivation of *E. coli.*

The synergy between temperature/heat and ultrasonication to enhance microbial inactivation is due to several factors. First, the intermolecular bonds of the liquid are weakened with increase in temperature; thus, cavitation bubbles are easily generated. When cavitation bubbles collapse, it generates enough energy to rupture the cell wall of the microorganisms [15, 25]. Second, the level of shear stress that a cell in suspension can withstand is decreased with increase in temperature [26], as temperature cause damage to cytoplasmic membrane [24]. A study made by Rooney (1972) on erythrocyte cells demonstrate that the level of shear stress withstood by the cell decreased by 55% or more when heated to 45°C or higher for 10 min. Lastly, the ability of cell to repair damage is inhibited by an increase in temperature. The rise in temperature impedes the cell to produce proteins that protects the cells against damage [16,26].

Allowing temperature to rise also affects the pH of the solution. In this study, the pH decreases with increase in temperature. A value of 6.48, average of four trials, was attained after 10 minutes of treatment. The value achieved is still within the set limit for *E. coli,* which is between 6.0 to 7.0. Therefore, the slight change in pH is not a factor that affects the inactivation of the microorganism.

The decrease in the pH of the solution is not brought about by the formation of hydrogen peroxide or free radicals, which is one of the products of cavitation, but by temperature. As the temperature increases the equilibrium constant also increases. In which case, results to the decrease of the pH of the solution. Again, this demonstrates the change in pH during ultrasonication is not a factor that influences the inactivation of the microorganisms. This finding is in agreement with the study conducted by Madge and Jensen (2002), where pH values ranged from 6.0 to 8.1, showed no significant relationship to disinfection. Moreover, a research conducted by Salleh-Mack (2006) showed lower pH value of 2.5 and 4.0 that significantly affects the inactivation of *E. coli.*

3.2 Sonication at variable amplitude

Amplitude was varied from 100% to 80%, 60%, and 40% for this stage of the experiment. Four trials were conducted for all amplitude. Varying the amplitude would alter simultaneously the power input of the probe. The power input was computed based from the energy dissipated, joules, per unit time. A digital LCD display provides a read-out of the joules delivered from the processor to the probe.

3.2.1 Effects of Power

In all trials, a linear relationship is evident for power and amplitude at exposure time fixed at 10 minutes. This denotes that power is directly proportional with amplitude. In addition, increasing the amplitude would increase the temperature. Consequently, this established a direct relationship between power, amplitude, and temperature. A maximum power of 31 W was attained at 100% amplitude (see Table 2). The rated power is the energy dissipated divided by the duration of the treatment using low frequency ultrasound.

Table 2
Summary of Energy Dissipated and Temperature at Variable Amplitude

% Amplitude	Temp, C	Energy, Joules					Power, W
		Trial 1	Trial 2	Trial 3	Trial 4	Average	
0	29.55	0	0	0	0	0	0.00
40	41.80	7336	7337	7339	7442	7364	12.27
60	47.50	10667	10663	10552	10531	10603	17.67
80	55.08	14325	14003	14199	14165	14173	23.62
100	61.20	18731	18512	18357	18398	18499	30.83

Inactivation of *E. Coli* is distinctly affected by amplitude as shown in Figure 5. At 100% amplitude, the inactivation rate is at 99.999% for 10 minutes exposure time. The lowest inactivation rate of 86.05% was achieved at 40% amplitude. Therefore, the higher the amplitude the greater is the inactivation. This result is in agreement with the studies made by Salamat (2005) and Reyes (2004), which showed that, inactivation of bacteria increases with increasing power.

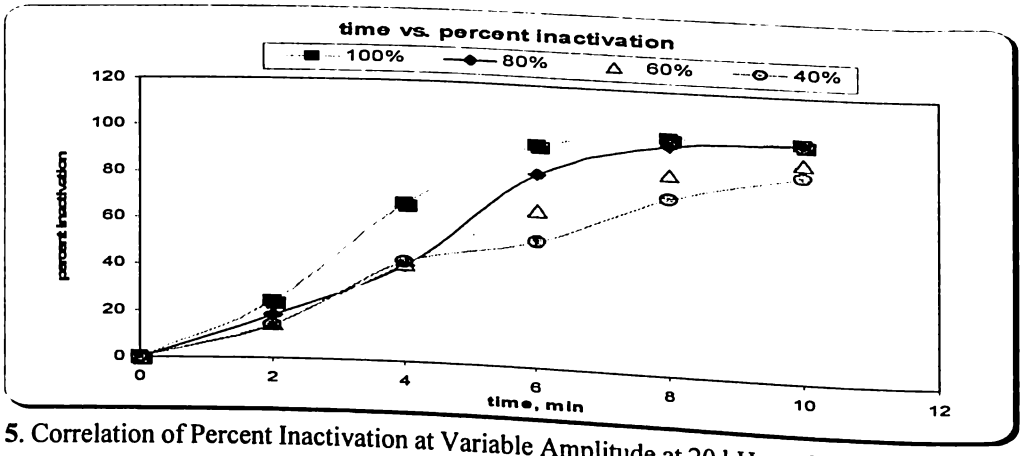

Figure 5. Correlation of Percent Inactivation at Variable Amplitude at 20 kHz and Sample Volume of 100 mL

The disinfection efficiency of several power inputs at fixed treatment time, about 10 minutes, was investigated using Chick-Watson law. Figure 6 shows that disinfection rate constants increased from 0.140 log kill/min at the lowest power (122 W/L) to 0.799 log kill/min at the highest power (310 W/L). The amount of inactivation in this study is close to the value obtained by Madge and Jensen (2003), where the disinfection rate constant is 0.83 min^{-1}. The difference would be explained by the power and media utilized. Their study made use of wastewater as media and the power input used in the experiments was 700 W/L. Use of wastewater would likely result to higher degree of cavitation, as expected with the presence of particles that would act as cavitation nuclei. With regards to the power employed, higher inactivation at higher power would result since more energy is being transferred into the water; thereby, subjecting the *E. coli* to more stress. According to Raso et.al. (1998), microorganisms can withstand high pressures but they are incapable of withstanding the quick alternating pressures produced during ultrasonic treatment.

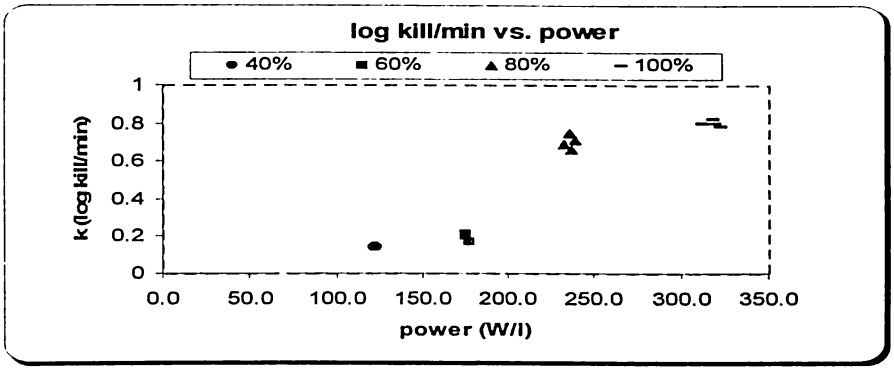

Figure 6. Disinfection Rate Constant vs. Variable Power at 20 kHz and Sample Volume of 100 mL

As for temperature, it also linearly increases with amplitude and time. Since elapsed time taken into account is only 10 minutes, the temperature change steadily increases. For all amplitude, temperature is one factor that aid in the inactivation.

According to Madigan (1997), *E. coli* has an optimum growth temperature of 39°C and maximum temperature range of 48°C to 60°C, before die-off. At 40% amplitude, the maximum temperature it attained for a 10 minutes exposure was only 41.80°C. This temperature can even be favorable to the growth of the *E. coli.* But due to the disruptive effect of the ultrasonic treatment, brought about by cavitation, inactivation was still feasible.

There are three mechanisms of ultrasonic treatment that contribute to its disruptive effect. First is the oxidant formation; wherein, water molecules disassociate into H- and OH- radicals and recombine to form hydrogen peroxide. The toxic effect of hydrogen peroxide on the bacteria enhances the inactivation. On the contrary, for low frequency ultrasound (20 – 100 kHz) the amount of oxidants form is relatively low [2,31]. Furthermore, according to Hua and Thompson (2000), high concentration of hydrogen peroxide is only evident at higher frequency. In addition, the increase in temperature would volatilize the hydrogen peroxide, further decreasing its concentration.

Second, ultrasonication produces a shock of high temperature and pressure. This shock disperses bacterial clusters making individual cells more susceptible to any change in the surroundings. Lastly, cavitation ruptures the cell wall, making them more exposed to disinfectants. For this study, the disinfectant would be heat. As explain before, heat/temperature not only decreases the fluidity of the cell membrane making it more vulnerable to shear stress, but decreases the ability of the cell to repair itself.

3.2.2 Effects of time

Effectiveness of biocidal inactivation is also influenced by time, power versus time resulted to a linear regression of 0.957. This is approximately equal to the study conducted by Madge and Jensen (2002); in which the value in their study is $R^2 = 0.951$. This suggests that Chick-Watson law is appropriate for describing the disinfection of water using ultrasound.

Figure 7 shows the relationship of power and time at 99.999% inactivation, the longer the exposure treatment the higher the percentage of inactivation. Furthermore, it distinctly shows that higher power input, at 310 W/L, was more effective for ultrasonic disinfection. Wherein, higher power in effect requires shorter contact time for treatment. This result is in agreement with the study made by El'piner (1964), that an increase in power is more efficient than a longer contact time for ultrasonic treatment. Hence, 100% amplitude is optimum for effective disinfection.

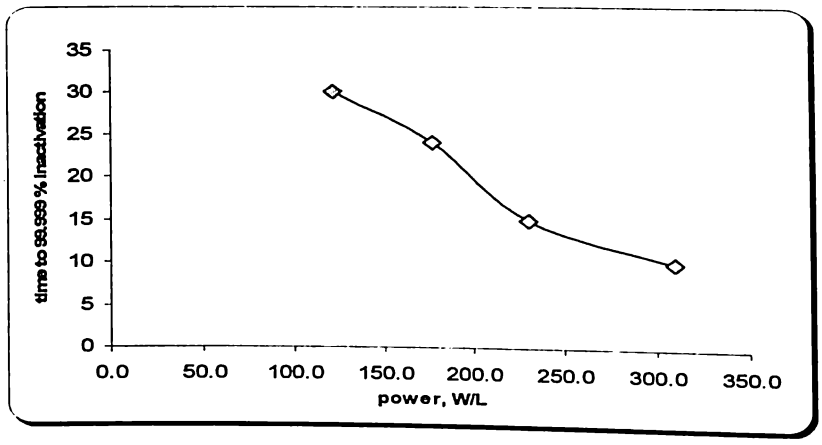

Figure 7. Power vs. Time to 99.999% or 5 log Inactivation using 20 kHz sonicator and Sample Volume of 100 mL at 20 kHz and Total volume of 100ml

3.3 Sonication at variable volume

The last stage of the experiment involves increasing the volume from 100 mL to 500 mL and 1000 mL. Having established the optimum power for inactivation, at fixed amplitude of 100% was used while varying the time from 5 to 30 minutes. This stage is comprised of three trials for each volume change. The effect of volume change is evident in Figure 8. As volume increases, temperature and percent inactivation decreases. The increase in volume reduces the concentration of the energy being dissipated. Therefore, it lowers the ability of cavitation bubbles to break down bacterial floc and the heat's biodical effect.

The maximum percent inactivation for 500 mL and 1000 mL are 98.30% and 97.35% respectively. This indicate that the energy input is optimized with 100 mL sample; in which, 99.999% inactivation was achieved after 10 minutes of treatment

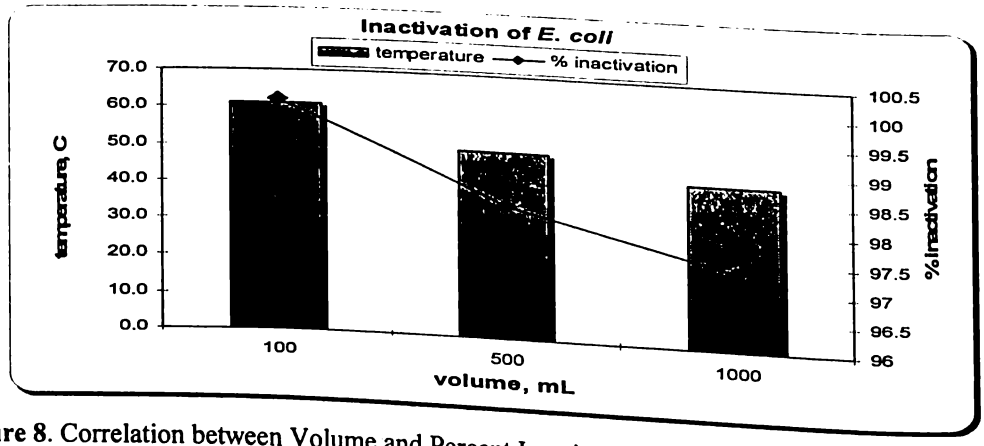

Figure 8. Correlation between Volume and Percent Inactivation at 20 kHz and Power input of 31W

Using the Chick – Watson law to graph Figure 9, it shows that increasing the volume would result to a first – order kinetic. The inactivation rate constant, $k_{e.coli}$, calculated for 100 mL, 500 mL and 1000 mL were 1.2747 min^{-1}, 0.1339 min^{-1}, and 0.1223 min^{-1}, respectively. Based from the result, a smaller k value signifies lower percent inactivation. This demonstrates how volume affects the rate of inactivation.

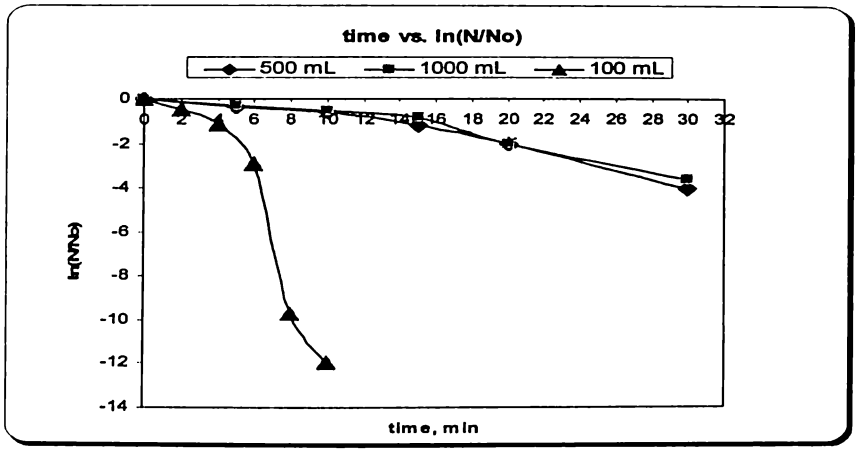

Figure 9. Correlation between Exposure Time and Inactivation of *E. coli* at 20 kHz and Power input of 31W

6. CONCLUSIONS

Inactivation of *Escherichia coli* using 20-kHz ultrasound unit in combination with elevated temperature has been investigated. From the experiments carried out in this research, the inactivation is directly proportional to temperature. About 5 log-reduction or 99.99 % inactivation was achieved at 61°C as compared to the 1 log-reduction at 41.8°C for 10 minutes of treatment. As presented, when sonication was used in conjunction with temperature high enough to cause thermal inactivation, about 50 – 60°C, a significant synergistic effect was observed. The synergy between temperature/heat and ultrasonication is due to the following: the intermolecular bonds of the liquid are weakened with increase in temperature; the level of shear stress that a cell in suspension can withstand is decreased with increase in temperature; the ability of cell to repair damage is inhibited by an increase in temperature.

The effects of power and volume change were also investigated in this research. This study showed that, increasing the power would result to higher percentage of inactivation at lesser contact time. The rated power of 31 W achieved 99.999% inactivation; while for the rated power of 12 W, percent inactivation was only 86.05% after 10 minutes of irradiation. The amount of sample utilized additionally affects the rate of inactivation. After 30 minutes of treatment, the log inactivation for the 100 mL, 500 mL and 1000 mL sample were 5 log-reduction, 1.7764 log-reduction, and 1.5794 log-reduction respectively. Therefore, for the ultrasonic processor employed in the research, the maximum volume for effective inactivation was at 100 mL. Based from the above results, the use of ultrasonication in combination with elevated temperature has the potential to become a technically viable alternative to traditional disinfection methods.

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