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SEQUENTIAL INACTIVATION OF *ESCHERICHIA COLI* IN DRINKING WATER USING LOW SONICATION FREQUENCY AND CHLORINATION

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ABSTRACT

It is known that ultrasound is able to inactivate bacteria and deagglomerate bacteria clusters or flocs through a number of physical, mechanical and chemical effects arising from cavitation bubbles. When sufficient power is applied, ultrasound alone is capable of killing bacteria. In addition, ultrasound at low power can also be used, in conjunction with other treatment, to improve its biocidal effect. Thus, the aim of this study was to investigate the effect of ultrasound treatment time operated at 20-kHz and 100% amplitude on the biocidal efficiency of chlorine towards Escherichia coli suspension.

For the biocidal effect of ultrasound, result showed a 100 % inactivation of E. coli was achieved when ultrasonic irradiation time was at 30 minutes. E. coli decay kinetics follow a first order reaction behavior with the value of inactivation rate constant $k_{E.coli} = 0.307\ min^{-1}$ (R^2 = *0.99). For primary disinfection with free chlorine, maximum reduction of E. coli colonies of 51.32 % has been achieved at 10 mg/L. However, there was no significant improvement in the disinfection effects of chlorine at two different concentrations of 10mg.min/L and 50mg.min/L. Furthermore, ultrasound in conjunction with chlorine significantly reduced the number of bacteria present and the amount of chlorine required for disinfection. The pretreatment by ultrasound was also proven to be more effective than pretreatment by chlorination.*

1. INTRODUCTION

Water is an essential component in the make-up of this planet and plays an essential role in supporting all life forms. When contaminated, however, it can transmit a wide variety of diseases and illness to man.

Chlorination, which has been widely applied for decontamination of water, has been causing the appearance of the resistance microorganisms such *Cryptosporidium* and *Fecal streptococci* [1]. As well as suffering from drawbacks on the formation of possible carcinogenic chemical by-products (Minear and Amy, 1996). Therefore, various physical and chemical techniques such as ozonation, ultraviolet light [2], ozonizing radiations (Kuruta *et al*, 2002) have been developed and used in water purification as an alternative of the conventional chlorination for dinsinfection purposes. However, they are very insufficient to eliminate the emerging chlorine-resistant microorganism.

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The destruction of microorganism by power ultrasound has been of considerable interest since the work of Havey and Loomis was first published in the early 1920's. In their study, they demonstrated that heating alone appeared to damage bacteria colonies but that ultrasound appeared to have greater effect. However, Harvey and Loomis predicted poor future for the commercial exploitation of sonication due to its high cost [3].

Recently, that situation has changed. Ultrasonic technology is more commonplace, costs have been reduced and applications are more economical. Power ultrasound can now be considered to be a viable alternative to conventional bactericidal techniques and noted as an effective technique for water treatment [4]. Furthermore, ultrasound treatment is an attractive and effective disinfectant of emergent pathogens such as *Cryptosporidium parum* that has high resistance against chlorination [1].

Ultrasound is able to inactivate bacteria and deagglomerate bacteria clusters or flocs through a number of physical, mechanical, and chemical effects arising from cavitation bubbles. The formation of cavitation bubbles leads to the generation of high pressure, high temperature, and locally and reactive free radicals (e.g. OH, $HO^{\cdot}_2$, and $O^{\cdot}$) that can cause cellular damage. The effects of ultrasound on microorganism are associated with the following mechanisms: *(1)* free radicals transfer into the bulk solution to attack the cellular membrane (a primary biocidal effect) and further a recombination of radicals to form an oxidant that acts as secondary biocide, *(2)* cavitation breaks up and disperses bacterial clumps and flocs to produce individual bacteria which are more susceptible to biocide, *(3)* shock wave damage to the cell wall which allows easier penetration of the biocide [3].

Because long contact and large power are required to achieve high rates of disinfection by ultrasound, current research on ultrasonic disinfection is focused on combining system with chemical processes to enhance the germicidal action of biocides and to reduce the chemicals requirements. It was shown that the ozone requirements for achieving high disinfection rates of *E.coli* were remarkably lowered with the use power of ultrasound in conjunction with ozone. The effect was attributed to increased ozone diffusion into the micro bubbles that creates a high gas-liquid surface area [4,5]. Moreover, some researchers have reported that sonolysis of UV irradiated TiO_2 suspensions in water using a 20-kHz ultrasonicator enhances the inactivation of *E. coli* through a synergy in hydroxyl radical formation [5].

Even with the above-mentioned study, little information on the combine effect of ultrasound and chlorination has been reported. Therefore, the aim of the study is to determine the effective ultrasonic treatment time in conjunction sodium hypochlorite solution towards the disinfection of *Escherichia coli* suspension, the indicator of microbiological contamination of the water supply. In addition, to ascertain whether sonication can lower the required chlorine dose for disinfection to ensure no by-products are formed at the end of the process. Lastly, to investigate whether the sequence sonication-chlorination is more effective than chlorination-sonication.

2. EXPERIMENT METHODS AND MATERIALS

2.1 Microorganism and media

E. coli ATCC 25922 Pure culture was obtained at the National Science Research Institute and was grown in a nutrient agar (Peptone: 5g; Beef extract: 3.0g; Agar: 15g and reagent grade water: 1 liter). Nutrient agar (NA) was prepared in screw-capped tubes in an inclined position and allowed cool. Then the NA slant was stored in a 4^0C refrigerator. One day prior to performing the

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disinfection experiment, the NA slants was inoculated with *E. coli* and placed in room temperature. The *E. coli* was allowed to grow overnight to stationary phase in the NA slant. The NA slant was suspended in distilled water tube and centrifuged to ensure the bacteria are dispersed, as well as to estimate the bacteria concentration per mL by Mc Farland standard test as initial count.

2.2 Materials

Free chlorine: A fresh stock of 1000 mg/l chlorine should be prepared prior to performing each disinfection experiment by diluting a 6% (v/v) sodium hypochlorite solution in water. The solution was then added with phosphate buffer to achieve the required chlorine dose and prepared before each trial.

Dechlorination solution: A 0.1N sodium thiosulfate solution, as dechlorination solution, was prepared from sodium thiosulfate crystal. The solution was then autoclaved prior to use.

2.3. Experimental set-up

The use of ultrasound: Ultrasonic processor of 20-kHz frequency, whose probe of 13 mm diameter was immersed to a 100 mL seeded aliquot, was operated at 100% amplitude in varying exposure times (1, 5, 10, 15, 20, 30 minutes).

The chlorination experiment: The seeded samples were contacted by different concentrations of free chlorine at varying exposure times. Residual chlorine levels of all the samples were measured by DPD Colorimetric Method.

3. RESULTS AND DISCUSSION

Current trend is towards the reduction in use of chlorine as disinfectant either by complete replacement with other biocides or by a reduction in the concentration required for treatment. Ultrasound should reduce the levels of chlorine requirement by the following reasons: (i) the destruction of bacteria cells by ultrasonic lysis, (ii) the de-aggregation of clumps of bacteria or other material trapping such bacteria to expose the 'masked" cells to disinfection.

3.1. Biocidal effect of ultrasound

The biocidal effects of ultrasound alone are shown in Figure 1. Approximately 50% of bacteria present were inactivated after only one minute of sonication. When the ultrasonic irradiation time was extended to 30 minutes, the maximum reduction of 4.1 log units of *E. coli* was achieved at a dose of 155 Wh/L. The observed results showed that bacterial inactivation increased with exposure time. However within 10 minutes of sonication, the graph reached the optimum point. Thus, it is not economical if the sonication time is longer than 10 minutes.

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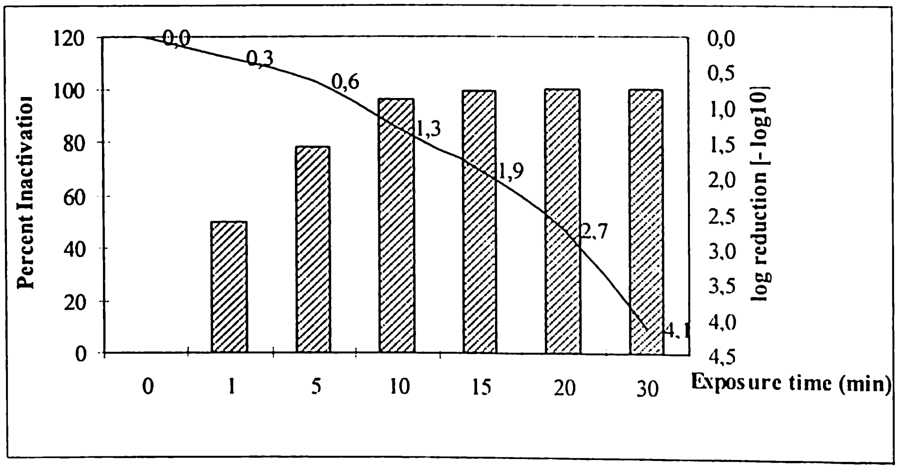

Figure 1: *Effect of sonication time on E .coli inactivation*

On the other hand, the inactivation of *E. coli* exhibits pseudo – first order behavior. As shown in Figure 2, at the applied ultrasound frequency of 20 KHz and 23.35 W/cm^2 of intensity, the value of inactivation rate constant k $_{E.coli}$ = 0.307 min^{-1}. It demonstrates that *E. coli* inactivation depends strongly on exposure time. Comparing to other pathogens, *E. coli* can be considered as non-persistent with the conventional disinfectants because of no spores forming and generally they are most sensitive to the environmental stresses. Hence, the cell structure of *E. coli* might be disrupted more easily by sonication even at low frequency and low power.

In a study by Hua (2000), the *E. coli* inactivation most likely resulted from a combination of physical and chemical mechanisms which occurs during acoustic cavitation. Thus, it is expected that higher intensities will enhance inactivation rates. However, for most processes, the increase in process rate does not continue indefinitely with higher sound intensities [4,8]. At ultrasound intensity of 30.13 W/cm^2, the value of $K_{e.col}$ = 0.11 min^{-1} and K_{strep} = 0.03 min^{-1} which was expected to be higher than the result of this study. The same result was observed by Scherba (1991).

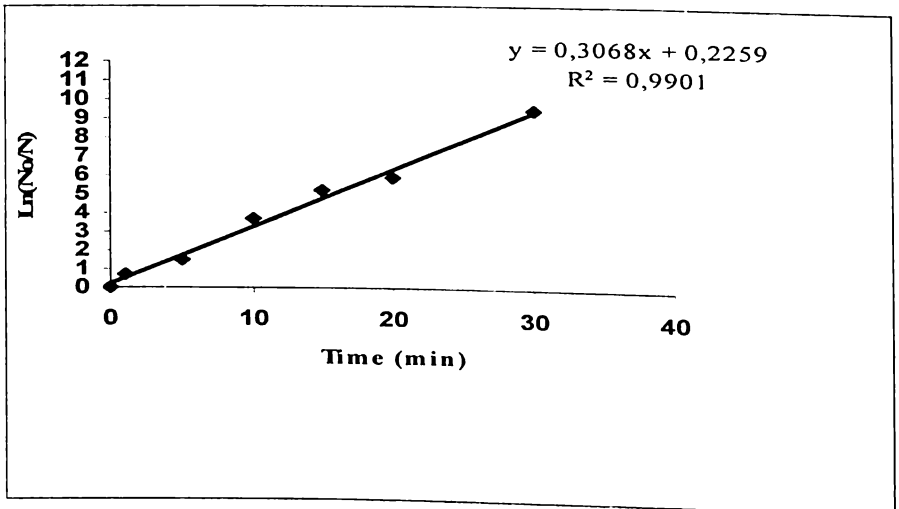

Figure 2: *Effect of sonication on E. coli inactivation Time vs.ln(No/N)*

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3.2. Biocidal effect of chlorine

The obtained results show that inactivation curve is characterized by pseudo – first order which is consistent with the observation of Rice (1999) at T = 25^{0}C and pH = 7. The correlative values of K at 1 minute and 5 minutes are 0.048 and 0.005, respectively. Comparing to K obtained in sonication part, the values of K of chlorination is much less, meaning that the higher efficacy was obtained for sonication.

Furthermore, *E coli* can be inactivated after exposure time. Figure 3 showed that the reduction of bacteria is relatively sharp at 0.5 ppm and 1 ppm. Maximum reduction of *E. coli* colonies of 51.32% were obtained at 10 mg/L of chlorine at 5 minute contact time while previous study done by Huang (1996) showed that 99% of killing effect on *E. coli* was attained when the required amount of free chlorine was 1.8 mg/l, for ClO_2, only 1.4 mg/L were required after 20 minute contact time at the same experiment condition.

However, in such as small range of contact time, there was no significant improvement in the disinfection effects of chlorine at two different dosages of 10 mg.min/L and 50 mg.min/L.

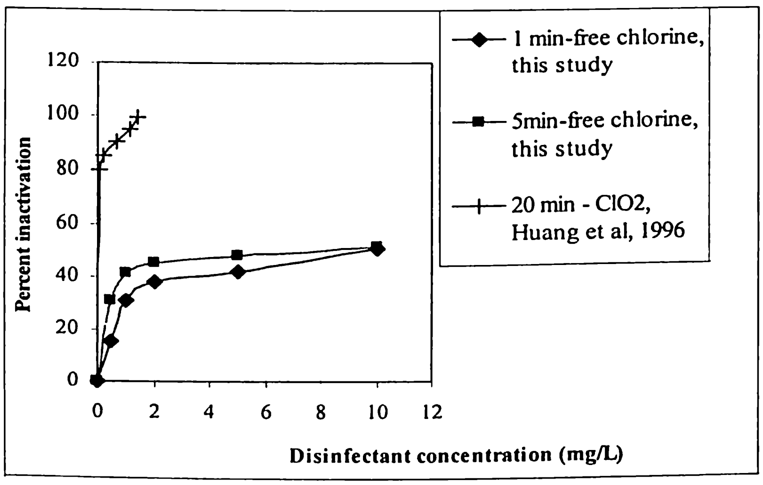

Figure 3: *Effect of chlorine dose on E. coli inactivation pH=7; T^0 = 20^{0}C*

3.3. The effect of sonication time on the biocidal action of chlorine

The results of the study on the combined effect of ultrasound and chlorination on the bacteria population are shown in Figure 4.

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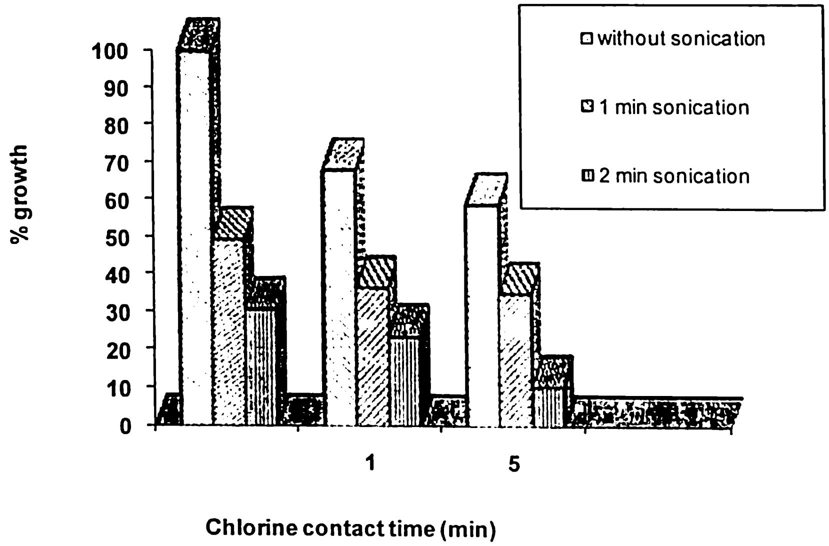

Figure 4: *Combined effect of ultrasound and chlorine (1ppm) upon bacteria growth*

As presented in Figure 4, the colonies of *E. coli* were only reduced by 31.95% with 1 min of chlorination alone. While 1 min of chlorination with ultrasonication showed an increased in the reduction of bacteria to 63% and 76% at 1 min and 2 min of sonication, respectively. Furthermore, when the chlorine contact time was increased to 5 minutes, as high as 89% of reduction of *E. coli* colonies were attained after 2 minutes of sonication.

Sonication was found to amplify the effect of chlorination. As shown in Table 1, the combined effect of sonication and chlorine is significantly better than sonication alone or chlorination alone.

Table 1: *Showing the synergistic effect of ultrasound upon bacteria growth (Power 31W, Free Chlorine: 1ppm)*

Treatment	Bacteria inactivated (%)
No treatment	0
Chlorine (1ppm)	31.95
Ultrasound	50.36
Combined (US + Cl_2)	
1 min sonication followed by 1 min chlorination	63.40
1 min sonication followed by 5 min chlorination	64.89
2 min sonication followed by 1 min chlorination	76.35
2 min sonication followed by 5 min chlorination	89.33

The above observation explained that ultrasound improves the effectiveness of chlorination through the dispersal of bacterial clumps, making individual bacteria more available to biocide attack. In addition, the bacteria are more permeable to the biocide by the temporary weakening of cell walls induced by sonication.

3.4 The effect of sequence of sonication and chlorination on bacteria action

The observed data show that pretreatment with sonication is more effective. The percent inactivation was computed to be 63.4% for 1 min of sonication followed by 1 min of chlorination, which is higher than that of 51.14% for 1 min of chlorination followed by 1 min of sonication. Otherwise, maximum percent inactivation of *E. coli* was as high as 89.33% for 2 min of sonication followed by 5 min of chlorination, while 71.4% of *E. coli* inactivation can be attained for 5 min chlorination followed by 2 min of sonication as shown in Table 2.

The sequence proved to be important in that when sample was sonicated prior to chlorination fewer bacteria survived comparing with sample was chlorinated and then sonicated. This observation can be explained by the degassing effect of ultrasound that removes any free chlorine from the solution and hence reduces the disinfectant concentration.

Table 2: *Showing effect of sonication and chlorination on bacteria inactivation*

Treatment	Bacteria inactivated (%)
Without treatment	0
1 min chlorination (1ppm) followed by 1 min sonication	51.14
1 min sonication followed by 1 min chlorination (1ppm)	63.40*
5 min chlorination (1ppm) followed by 2 min of sonication	71.40
2 min sonication followed by 5 min of chlorination (1ppm)	89.33

4. CONCLUSIONS

Ultrasound can be considered a viable alternative to conventional bacteriocidal techniques as an effective technique of water treatment. In addition, ultrasonicator in combination with chlorination is expected to be a promising method for inactivating the pathogens in drinking water.

From above studies on the effects of ultrasound on disinfection on water, Ultrasound is seen to be an appropriate method for water disinfection and study showed the promising results as follows:

1. Percent inactivation of *E. coli* can reach 100% when ultrasonic irradiation time extends to 30 minutes. *E. coli* decay kinetics follow a first order reaction behavior with value of inactivation rate constant $k_{E.coli} = 0.307\ min^{-1}$
2. Ultrasound reduces the amount of chlorine required for disinfection; a chlorine concentration of 1 mg/L can be used effectively.
3. Maximum reduction of *E. coli* colonies of 51.32% has been obtained at 10 mg/L of chlorine at pH=7 and $T^0 C = 25^0C$

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4. There was no significant improvement in the disinfection effects of chlorine at two different dosages of 10 mg.min/L and 50 mg.min/L at pH=7 and $T^0C = 25^0C$
5. The maximum effects of ultrasound on biocidal action of chlorine are achieved within 2 minutes of sonication followed by 5 minutes of chlorination
6. Sonication was seen to amplify the effect of chlorination and the combination is significantly better than sonication alone.
7. The effect of sequence of sonication and chlorination on biocidal action was observed and proved to be important in that the pretreatment by ultrasound can inactivate more colonies of *E. coli* than pretreatment by chlorination.

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