Flow Cell Ultrasound Treatment: The Influence of Sonication Media and Temperature on the Disruption of Escherichia coli Wild Type Cells

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Abstract: This study was conducted to discover ultrasonic treatment as an innovative technology specifically for Escherichia coli wild type cell disruption for the disinfection process. Therefore, this study aims to evaluate the influence of process conditions through the performances of Flow Cell Ultrasound (FCUS) as the bacterial disruption. In order to determine the influence of the process condition through flow cell ultrasound, the research approach used in this study was measured by the disruption of Escherichia coli cells with inoculum (10^6 CFU/mL), sonication media Phosphate Buffer Saline (PBS) and peptone water, 35% amplitude and flow rate (70 mL/min). There was statistically a significance difference (p<0.05) between peptone water and PBS where PBS as the sonication media showed 6.93% higher cells disruption as compared to the peptone water. The percentage removal at 120 min treatment for cells disruption exhibited 98.55±2.51% these interpreted that the disruption of Escherichia coli wild type cells comply with first-order kinetics model and k = 0.0353 min⁻¹. Furthermore, ANOVA elucidated that the temperature increases by time and p<0.05 and resulted in an average final temperature of 28.6°C which was within the non-lethal temperatures for Escherichia coli. Thus, the use of flow cell ultrasound was identified to be beneficial uses for water disinfection due to green process occurred during their removal performances.

Key words: Flow cell ultrasound, Escherichia coli wild type cells, bacterial disruption, sonication media, temperature, technology

INTRODUCTION

In the last few years, a growing awareness of inadequate access to clean drinking water and sanitation has been witnessed. Innumerable conventional methods have been applied for the disinfection process in the aspect of pathogen removal. These include chlorination, ozonation and Ultraviolet Radiation (UV) (Al-Juboori and Yusaf, 2012; Betancourt and Rose, 2004; Doosti et al., 2012; Tansel, 2008). However, there are purification limitations and application problems for instance, the generation of toxic secondary pollutants such as THMs and brominated haloacetic acids (Doosti et al., 2012; Budari et al., 2015; Zhou et al., 2016). In the coming decades, problems related to water are expected to grow worse. Hence, the ultrasonic treatment as new innovative technology as an effective and non-toxic method is urgently needed in water treatment industry is highly prerequisite since, it will help to produce an eco-friendly technology for microbial pathogen removal (Budari et al., 2016; Zhou et al., 2016a, b). Ultrasonic irradiation may inactivate microorganisms in several mechanisms that are based on the acoustic cavitation. This lethal effect is due to the extreme pressure variations caused by rapid formation, growth and amount of energy released which will result in the occurrence of bubbles collapse. These extreme conditions could mechanically damage the bacterial cell walls and contributes to the disinfection process and consequently, the formation of heat generation (Doosti et al., 2012; Gibson et al., 2008).

Ultrasonic technology has been used as a non-chemical approach and acts as an innovative technology which has been regarded as a highly potential technology in the removal of microbial from water and wastewater treatment industries (Andaluri et al., 2012). Hence, an ultrasonic treatment is expected to produce ample finding that minimises and reduces the negative impacts on human health. Therefore, the objective of this research is to investigate the low intensity FCUS treatment for the disruption of Escherichia coli wild type cells and to determine the influence of sonication media and temperature. Specific objectives include the following: to compare the performances of FCUS as Escherichia coli

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disruption in PBS and peptone water, to determine the thermal effects on the reduction of model organism.

MATERIALS AND METHODS

Description of reactors set-up: Low intensity Flow Cell Ultrasound (FCUS) (1.03 W/cm²) was constructed as a continuous loop flow process as a function for greater performances as consequences with residence time. An inoculated sample was fully sonicated by transducers as seen in the schematic diagram of the experimental setup (Fig. 1). The technicality of ultrasonic was operated at 30 kHz fixed frequency with an amplitude of 35% to the system and 50 min residence time. One inlet for the sample influent and one outlet for the treated sample were designed at the flow cell ultrasonic. The volume of inoculated samples involved was 50 mL and placed in 500 mL sterile beaker and 0 time sample was measured.

The nozzle was fabricated by a piezo-ceramic technology as a driver for piezoelectricity and function such as the production of electronic frequency generation (Budari et al., 2015). The FCUS reactor was sterilised in 70% alcohol for 15 min and rinsed by sterile ultrapure water for 15 min prior to the sonication of the sample in order to avoid contamination during the sonication process (Gera and Doores, 2011). The experimental system included the Bunsen burner as a function for the sterilisation area in a radius of 10 cm from the centre of the flame to the flow cell ultrasonic. The temperature during sonication never exceeded the lethal conditions with a maximum temperature of 28.6°C.

Escherichia coli wild type cells cultivation and inoculum preparation for sonication: Experiments were conducted on laboratory scale using Escherichia coli wild type cells isolated bacteria from College Mawar Wastewater Treatment Plant, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor, Malaysia (Budari et al., 2015). Escherichia coli is known as a sub-group of faecal coliform and is employed as an indicator bacteria for water contamination. By using Escherichia coli wild type cells isolated bacteria as model organisms hugely suggest that FCUS is adaptable to real environment disinfectants (Zhou et al., 2016). The cultures were activated by a loopful (10 mL) of glycerol bacterial stock that was streaked in Eosin Methylene Blue (EMB) agar and incubated at 35-37°C for 24 h. Hence, after incubation, the bacteria will metabolise the lactose by producing acid by products in the media, resulting in bacterial growth with dark blue black and green metallic sheen in colour (Fig. 2). Meanwhile, for the inoculum preparation, Escherichia coli activated cultures with specification of

![Flow cell ultrasonic experimental systems](image)

**Fig. 1:** Flow cell ultrasonic experimental systems

![Pure culture of Escherichia coli in Eosin Methylene Blue (EMB) agar](image)

**Fig. 2:** Pure culture of Escherichia coli in Eosin Methylene Blue (EMB) agar

2.0 mm diameter and 2 colonies from EMB agar were cultivated in 10 mL nutrient broth with an incubation of 18 h. From the result, this fermentation produced 10⁶ CFU/mL inoculum concentrations. The resulting inoculum with a volume of 5 μL was added to 499.995 mL sonication media to produce 10⁶ CFU/mL cells. The final volume was 50 mL and 10⁶ CFU/mL bacterial cells inoculated engendered a sonication sample.

Sonication media: To determine the influence of sonication media on ultrasound as bacteria disruption isonic saline (phosphate buffer saline) at pH 7.44±0.01, Oxoid, Hampshire, United Kingdom (BR0014)) and peptone water Oxoid, Hampshire, United Kingdom (CM0009) were used as the sonication samples with a total volume of 50 mL. For determining the effect of sonication media on flow cell ultrasound, the number of bacteria was 10⁶ CFU/mL Escherichia coli Wild Type Cells.

Enumeration of bacteria: Aliquots were serially diluted in peptone water (sterilised by autoclaving at 121°C for 15 min) and were spread plate in triplicates on EMB agar
plates (Salleh and Roberts, 2007; Reyes, 2011). The plates of one serial dilution \(10^{-1}\) were incubated at 35-37°C for 24 h and CFU/mL counted. Survival *Escherichia coli* wild type cells (initial number of the cells-number of live cells after ultrasonic irradiation) were triplicate counted and averaged as percentage removal. These procedures were the same for all experiments.

**Treatments without temperature control:** The FCUS treatments without temperature control consisted of simulated PBS as the inoculated sample. Measurement of the temperature influence was conducted at 30 kHz fixed frequency with an amplitude of 35% to the system. Temperature measurements were recorded for every 10 min intervals using multiparameter XL 60 (Fisher Scientific, Hampton, New Hampshire, United States). All experiments were performed in triplicates and the error bars in the figures indicate the standard deviations. The metal probe of the temperature measurement was sterilised in 70% alcohol and dried by flame in order to evade contamination during the experiment process (Gera and Dooree, 2011).

**Statistical analysis:** A statistical analysis was conducted by using IBM SPSS Statistics 24 Software. The data analysis was performed using ANOVA and the probability value involved is <0.05.

**RESULTS AND DISCUSSION**

**Influence of sonication media on disruption of *Escherichia coli* wild type cells:** A regression analysis was conducted to determine the influence of sonication media on the disruption of *Escherichia coli* wild type cells. There was a statistically significant difference between peptone water and PBS at 0.05 levels as determined by the regression analyses with ANOVA for the disruption of cells at \(p<0.05\) and higher coefficient determination \(R^2\) where PBS acted as an inoculated sample for higher disruption at 50 min sonication time (Mean±SD: 86.64±1.60%, \(R^2\) 0.995) as compared to peptone water (Mean±SD: 79.71±6.96%, \(R^2\) 0.972). The curve was fitted to quadratic graph forms that represented the relationships between time and percentage removal (Fig. 3).

The effect of FCUS for the disruption of *Escherichia coli* by flow cell ultrasound increased when there was a rise in the residence time through the continuous loop reactor. These observations can be interpreted as a consequence of a proportional relationship between time and microorganism disruption with the sonication media effect (Guzel et al., 2014; Liu and Chiu, 2005; Pilli et al., 2011). Peptone water has less influence to the disruption of these bacteria by FCUS as compared to PBS by suggesting the presence of hydrolysis of protein this may lead to the increase of the resistance of bacteria to disrupt ultrasound treatment by supplementing minimal nutrient to the bacteria (Garcia et al., 2010). Hence, from the result, PBS was employed as the sonication media for further exploring FCUS as bacterial disruption.

**Disruption of *Escherichia coli* wild type cells:** This research presented an ultrasound reactor with flow cell arrangements and this suggested more flexibility for a large-scale industrial application. The ultrasound reactors arranged the multiple point sources of transducers on the cell wall of the reactor on the opposite faces, so that, standing wave patterns can be generated (Fig. 1) (Gogate et al., 2011). Performing the ultrasound application may cause the death of microorganisms with sufficient frequency, power and irradiation time (Pilli et al., 2011; Mahvi et al., 2005; Yusuf and Al-Juboori, 2014). Higher cells disruption was highlighted by the previous literature correspondingly with less power intensity, less oscillation amplitude and less frequency (25-40 kHz). As consequences of the enlargement of the surface area through the cells irradiation, the energy dissipation over the sonication media would exaggerate the area in flow cell ultrasound, hence, there is a larger disruption of cells at 30 kHz fixed frequency, amplitude of 35% and flow rate of 70 mL/min (Budari et al., 2015).

There is a statistically significance difference between time and the cell disruption at 0.05 levels as determined by
one-way ANOVA (p<0.05). The percentage removal at 120 min treatment for *Escherichia coli* wild type cells disruption exhibited a non-linear graph ($R^2$ quadratic = 0.957) in PBS (Fig. 4). The disruption of cells was relatively rapid during the first 10 min (37.68%) of the sonication process. At the end of 120 min, the percentage removal of the population was calculated to (Mean±SD: 98.55±2.51%). Even though the disruption increases by time, the trend removal was reduced the rapid disruption and generated a tailing. A tailing phenomenon during the treatment attributed to the presence of more resistant cells in the inoculated samples. Tailing is expected to cause clumping and aggregation of bacterial death cells at acoustic pressure nodal planes where cavitation is absent due to the standing wave formation (Gera and Doores, 2011).

Figure 4 reveals that the coefficient determination $R^2$ linear = 0.997; these interpreted that the disruption of *Escherichia coli* wild type cells comply with first-order kinetics model, $k = (\ln(N_0/N))/t$ where k is the first-order rate constant and it is estimated from the slope by plotting $\ln(N_0/N)$ versus time as shown in Fig. 4. Where, $N_0$ is the bacteria population at $t = 0$ and N is the live bacteria after irradiation. First-order disruption kinetics have also been reported in effluent wastewater (Neis and Blume, 2003). At the applied ultrasound flow rate of 70 mL/min and amplitude of 35%, the first-order rate constant was 0.0353 min$^{-1}$.

**Influence of temperature:** The treatments were conducted without temperature control and ambient conditions of pressure (Fig. 5). Temperature measurements were recorded every 10 min and all measurements were recorded in triplicates. A statistical analysis was conducted to determine the significant difference between time (0, 10, 20, 30, 40 and 50 min) and cell disruption effect with temperature consequences. The analysis of variance (ANOVA), interpreted that the temperature increases by time and $p<0.05$. Resulting from the descriptive analysis, the Standard Error (SE) of the mean for the initial temperature ($t = 0$) was 1.55 and is higher than other time's SE of the mean (0.23, 0.17, 0.36, 0.44 and 0.45 equally by the increases of time, respectively). This situation suggested that an ambient temperature of inoculated sample was to be stored at a refrigerator compartment (1-6°C) and under queued before the treatment process hence, this caused the reduction of temperature to 18.7°C (Fig. 5). After 10 min of sonication, the temperature increased with a lower value of SE and the results were more precise. The experimental results produced an average final temperature of 28.6°C which was within the non-lethal temperatures for *Escherichia coli* (Salleh and Roberts, 2007). At a first glance, the results seemed contradictory to the report of Rasol et al. (1999) who reported that the starting temperature of inoculated media had a noticeable effect on the power generated to the cells. However, the initial temperature was raised, a higher SE of the temperature has no effect to the increase of the temperature after 10 min sonication and as an implication there was no effect to the increase of heat generation. The energy input from the flow cell transducers is transferred into mechanical energy such as friction, turbulences, waves and cavitation. Thus, the temperature significantly increased which attributed to the discharge waves.
generated from the cavitation zone of the liquid phase as an effect of heat generation during the sonication process (Salleh and Roberts, 2007; Olvera et al., 2009).

CONCLUSION

There was a statistically significant difference between peptone water and PBS as determined by the regression analyses with ANOVA for the disruption of cells (p<0.05) and higher coefficient determination (R²) where PBS acted as an inoculated sample of higher disruption at 50 min sonication time (Mean±SD: 86.64±1.60%, R² 0.995) as compared to peptone water (Mean±SD: 79.71±6.96%, R² 0.972).

The percentage removal at 120 min treatment for Escherichia coli wild type cells disruption exhibited a nonlinear graph (R² quadratic = 0.957) in PBS. At the end of 120 min, the percentage removal of the population was calculated to (Mean±SD: 98.55±2.51%). The R² linear = 0.997; these interpreted that the disruption of Escherichia coli wild type cells comply with first-order kinetics model and the k value was 0.0353 min⁻¹. Furthermore, the Analysis of Variance (ANOVA) interpreted that the temperature increases by time and p<0.05 and resulted in an average final temperature of 28.6°C which was within the non-lethal temperatures for Escherichia coli.

In consequence, flow cell ultrasound is capable to intensify the application with significance effects of sonication media, temperature and duration of sonication. Thus, presently, flow cell ultrasound has conclusively been proven as one of the valuable uses for water disinfection, due to the greenery technology on their removal performances.

ACKNOWLEDGEMENT

This research is financially supported by the Research Acculturation Grant Scheme (600-RMI/RAGS 5/3 (151/2014)) from the Ministry of Higher Education (MOHE), Malaysia.

REFERENCES


