Evaluation of Power Effect on Disruption of Escherichia Coli Wild Type Cells in Flow Cell Ultrasound Treatment

Budari, N.M. *, Ali, M.F., Ku Hamid, K.H., Khalil, K.A., Zainal, S., Musa, M.

Abstract: ***Flow cell ultrasound treatment used non-chemical action and was conducted with room temperature and ambient conditions of pressure for disruption of cells, allowing the consideration of ultrasound as a clean technology. The 30 kHz flow cell ultrasound that was demonstrated technically at 55 ml/min and 35 % power increments had higher performances, with (i) 94.59 % model organism disruption and (ii) the lowest cost of treatment at 0.0579 kWh/liter of electric energy per order and equally to 2.90 USD/m3. Furthermore, there was a statistically significant difference by one-way anova between flow rates and power effect in flow cell ultrasound (p < 0.05), where Tukey Honest Sig-nificant Difference (HSD) post hoc analysis revealed that the increase of flow rate from 40 to 70 ml/min was generating increases of power from 0.00336 to 0.00409 kW. Thus, flow cell ultrasound is an efficient sustainable technology but the economic costs need to be carefully balanced with the need for sustainable treatment for future.***

Index Terms: Flow Cell Ultrasound, Escherichia Coli, Power, Electric Energy per Order, Monetary Cost,

I. INTRODUCTION

The currently employed disinfection technologies are chlorination, ozonation, and ultraviolet radiation (UV) [1]–[5]. But, these treatments have some restrictions and application difficulties such as DBPs and may cause skin cancer on humans [6], [7]. In recent years, it has been established that ultrasonic technology has been used as a non-chemical approach and acts as an attractive green disinfection process which has been regarded as a highly potential technology which function as bacterial disruption for water and wastewater systems [5], [8].

Ultrasonic irradiation may inactivate microorganisms in several mechanisms that are based on the acoustic cavitation. This cells 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 in extremely small interval milliseconds of time in a liquid phase. These extreme conditions could mechanically damage the bacterial cell walls and the effects of flow cell ultrasound reactor expecting expected cells damages restricted to the radial distances near the emitting area and finally contributes to the disinfection process [3], [9], [10].

Additionally, the performance of the reactor depended on the variables that may lead to the improvement of process efficiency. Hence, the evaluation of time, power increment and flow rate was complemented as an independent variable and established the direction of the performance of flow cell ultrasound reactor. Therefore, the objective of this study is to evaluate the power effect as a dependent variable for the *Escherichia coli* wild type cells disruption. Specific objectives include the following: (i) to investigate the performances of FCUS as *Escherichia coli* disruption at 40, 55, and 70 ml/min in 14, 28, 35, 42, 50, 64, and 92% of power increments through the disruption model organism; (ii) to evaluate the energy consumption and monetary costs of flow cell ultrasound for the treatment of the inoculated samples.

II. MATERIALS AND METHODS

2.1 Construction of the Pilot Scale Water Disinfection System This disinfection system consists of five subsystems namely; (i) sterile water container with 50 ml of inoculated samples, (ii) peristaltic pump (Major Science, USA), (iii) analog magnetic stirrer plate (Fisher Stirrer, Waltham, USA) with combination sterilisation magnetic stirring bar with the length of 20 mm, and constant stirring knob at 1 to mix the inoculated samples well, (iv) Bunsen burner as a tool for the sterilisation area in a radius of 10 cm from the centre of the flame to the flow cell ultrasound (Campingaz with cartridges C206), and (v) disinfection unit with 30 kHz fixed frequency custom ultrasound and digital generator was used to analyse emitted frequency and output power (Sonaer Ultrasonics, New York, USA). One inlet for the sample influent and one outlet for the treated sample were designed at the disinfection unit. The flow cell ultrasound emitting surface and silicon tubing in both internal and external were sterilised in 70% alcohol for 15 minutes and rinsed with sterile ultrapure water for 15 minutes prior to the sonication of the sample in order to avoid contamination during the sonication process [5], [11]. A generator was used to supply power with different levels of output (0 to 100% and every increment at 7%). The data sets were involved for the high flow rate of treatment (40, 55, and 70 ml/min).

Revised Manuscript Received on December 30,2018.

Budari, N.M., Faculty of Civil Engineering, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, MALAYSIA,

Ali, M.F., Faculty of Civil Engineering, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, MALAYSIA,

Zainal, S., Faculty of Chemical Engineering, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, MALAYSIA.

Musa, M., Faculty of Chemical Engineering, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, MALAYSIA.

Ku Hamid K.H., Faculty of Chemical Engineering, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, MALAYSIA,

Khalil, K.A., Faculty of Applied Science, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, MALAYSIA.

The maximum available ultrasonic power delivered to the flow cell ultrasound was 100% power increment.

But, for the reactor's safety factor, the maximum power increment for the evaluation was 92% [12]. For further flow cell ultrasound, the power increments at 14, 28, 35, 42, 50, 64, and 92 (%) were selected to analyse the evaluation of flow cell ultrasound with 60 minutes residence time.

2.2 *Escherichia coli* Wild Type Cells Cultivation and Inoculums Preparation for Sonication

Experiments were conducted on a laboratory scale using *Escherichia coli* wild type cells isolated bacteria from College Mawar Wastewater Treatment Plant, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor, Malaysia [5], [13]. The cultures were activated by using 10 µl of glycerol bacterial stock that was streaked in Eosin Methylene Blue (EMB) agar and incubated at 35 – 37 ^{o}C for 24 hours. Meanwhile, for the inoculum preparation, *Escherichia coli* activated cultures with specification of 2.0 mm diameter and 2 colonies from EMB agar were cultivated in 10 ml nutrient broth with an incubation of 18 hours. From the result, this fermentation produced 10^8 CFU/ml inoculum concentrations and by dilution process the final volume was 50 ml and 10^4 CFU/ml bacterial cells in the sonication sample.

2.3 Analysis Enumeration of Bacteria

Aliquots were serially diluted in peptone water (sterilised by autoclaving at 121°C for 15 minutes) and were spread plate in triplicates on EMB agar plates (Merck, Darmstadt, German) [14], [15]. The plates of 10^{-1} serial dilution were incubated at 35 – 37 ^{o}C for 24 hours and CFU/ml counted. The surviving *Escherichia coli* wild type cells (initial number of the cells at zero time - number of live cells after sonication treatment) were triplicate counted and averaged as percentage removal. These procedures were the same for all experiments.

III. STATISTICAL ANALYSIS

All experiments were carried out with repeatability at least in duplicate. A statistical analysis was conducted by using the IBM SPSS statistics 24 software. The data analysis was performed with the probability value of less than 0.05.

IV. RESULTS AND DISCUSSION

3.1 Effect Factor of Power Dissipation

The experiment was conducted via flow cell ultrasound with an advantage on the reactor design whereas an emitting area was fully sonicated in the inoculated samples treatment area. The performances of flow cell ultrasound was analysed using the statistical analysis containing one dependent variables; (i) *Escherichia coli* cells disruption, meanwhile, the two independent variables involved were (i) flow rate (ml/min) and (ii) power increments (14%, 28%, 35%, 42%, 50%, 64% and 92%). Results of the statistical analysis may assist in the selection of the power output evaluation against the *Escherichia coli* wild type cells disruption. Figure 1 shows the 3D bar graph for the effect of flow rate and power increments versus *Escherichia coli* disruption with 3D rotation in specification of vertical (10) and horizontal (210).

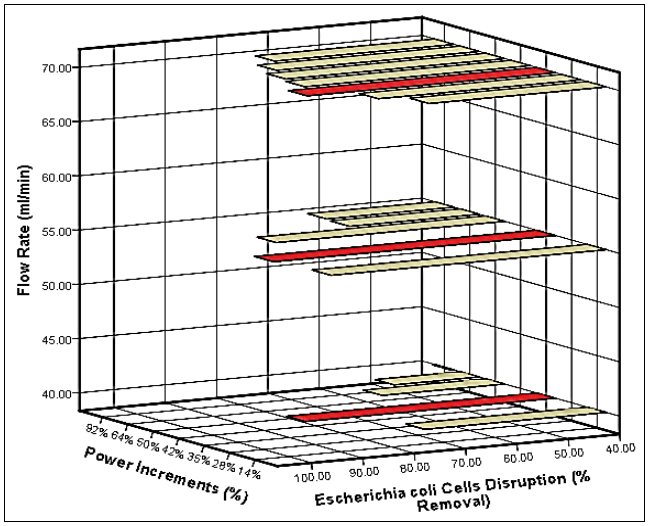

Figure 1. Effect of *Escherichia coli* cells disruption in concentration (10^4 CFU/ml), flow rate (40, 55 and 70 ml/min), power increments (14, 28, 35, 42, 50, 64 and 92%) at 60 minutes sonication time

The design limitation of flow rate for flow cell ultrasound was approximately at 70 ml/min. From the reactor design factor, power to the nozzle is configurable in 7% increments. Hence, at flow rate 70 ml/min, the critical analysis was conducted at 28%, 35% and 42%. These analyses were carried out to ensure that 35% of power increment is the best performances of sonication reactor (88.40 % removal). These trends were followed by 55 and 40 ml/min and it also confirmed that the power increment at 55 ml/min was the most excellent flow rate (94.59 % removal) and 40 ml/min was 88.80 % removal. Higher cells disruption was highlighted by the previous literature correspondingly with less power output consequently less power intensity, less amplitude, and less frequency (25 – 40 kHz). As for the 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, 35% of power increments at flow rate of 40, 55 and 70 ml/min [5], [13], [16].

3.2 Energy Consumption and Monetary Cost

The key aspect of an efficient flow cell ultrasound reactor is the effect of consequent power output to the disruption with minimum energy consumption [8]. The increasing percentage of power from 0% to 100% consequently increases the amplitude in the flow cell ultrasound. Electric energy per order (EE/O) is the electric energy in kilowatt hours (kWh) required for the disruption of pathogenic contaminants by one order of magnitude in a unit volume of inoculated samples [8], [17]. The EE/O was calculated using Equation 1, where P is power (kW), t is time (h), V is the volume of inoculated samples (liter), and C_i and C_f are the initial and final concentrations of cells in CFU/ml respectively.

$$E_{EO} = \frac{P \, x \, t}{V \, x \log\left(\frac{C_i}{C_f}\right)} \quad (1)$$

Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication

One-way ANOVA was conducted to analyses a significant value between flow rate as an independent variable and power as a dependent variable. There was a statistically significant difference between flow rates and power effect at 0.05 levels as determined by one-way ANOVA in flow cell ultrasound ($p < 0.05$). The Tukey HSD analysis was performed in order to get more conformist and less conformist estimations of these differences [18]. From the homogeneous subsets by Tukey HSD post hoc analysis, it was found that the increase of flow rate at 40, 55 and 70 ml/min was generating increases of power from 0.00336, 0.00378 and 0.00409 kW respectively (Table 1).

The key indicators of performance flow cell ultrasound was constitute to the design of the reactor where flow rate preceded than the power increments during the operational of sonication process. From the results that have been explored the cells disruption does not directly proportional to the power dissipation needed during the treatment process. Instead, the cells disruption was reached the pick of performance at flow rate 55 ml/min and power dissipation 0.00378 kW and then for further increases of power dissipated in the system at 0.00409 kW resulted reduces of cells disruption. This was attributed to the formation larger bubbles clouds and consequently to be shield during the irradiation process [19], [20].

Table 1. Electric energy per order for 10^4 CFU/ml *Escherichia coli* disruption by 30 kHz flow cell ultrasound treatment

Flow rate (ml/min)	40	55	70
Power (kW)	0.00336	0.00378	0.00409
Time (hours)	1	1	1
Volume (liter)	0.05	0.05	0.05
Log (C_i/C_f)	0.9512	1.3054	0.9192
EEO (kWh/liter)	0.0707	0.0579	0.0889
Cost in Malaysia per kWh (RM)	0.218	0.218	0.218
Cost in Malaysia per kWh (USD)	0.05	0.05	0.05
Cost of treatment (RM/m^3)	15.41	12.62	19.38
Cost of treatment (USD/m^3)	3.54	2.90	4.45

The monetary cost to complete of 60 minutes at 0.05 liter capacity of treatment by ultrasound reactor was shown in Table 1. The table observes the influence of flow rate was affecting different power. Thus, from this study the influence of monetary cost and EE/O was highly related to both process and design parameters/variables such as flow rate and disruption of pathogenic contaminants as a function and decisive direction for performance of flow cell ultrasound [8]. The higher performance of reactor by cost per Malaysian rate for operating 30 kHz flow cell ultrasound was 55 ml/min flow rate and 10^4 CFU/ml inoculated *Escherichia coli* wild type cells. The lowest cost of treatment was approximately 0.0579 kWh/liter of electric energy per order and 2.90 USD/m^3. This cost is lower as compared to EE/O 0.0707 and 0.0889 kWh/liter at 40 and 70 ml/min respectively (Figure 2). Based on the energy consumption, flow cell ultrasound sonication at 55 ml/min flow rate and 35 % power increments as a consequence at 94.59% cells disruption would be more cost effective and increase energy efficiency. However, the requirement for high energy consumption unfortunately remains as an important limitation for ultrasonic application to water and wastewater treatment systems and industrial application [17], [21], [22]. Thus the economic costs need to be carefully balanced with the need of sustainable treatments for the future, especially on energy influences such as power, time, and volume.

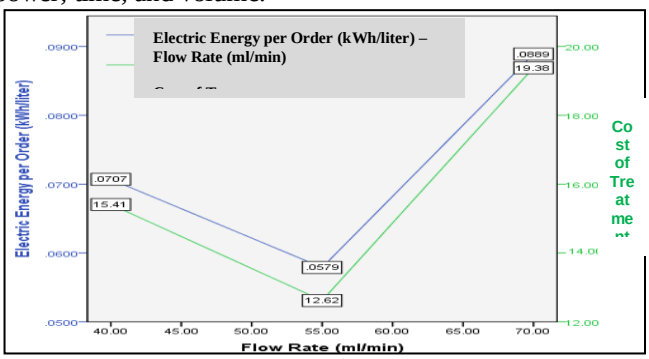

Figure 2. Effect of flow rate (40, 55 and 70 ml/min) on the electric energy per order and cost of treatment

V. CONCLUSION

In conclusion, 30 kHz FCUS demonstrated technically at 55 ml/min and of 35 % power increments as a higher performance where (i) 94.59 % model organism cells disruption and (ii) the lowest cost of treatment at 0.0579 kWh/liter of electric energy per order and equally to 2.90 USD/m^3. Thus, flow cell ultrasound is an efficient sustainable technology towards increasing energy efficiency.

VI. ACKNOWLEDGEMENT

This work 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

1. R. A. Al-Juboori and T. F. Yusaf, "Improving the performance of ultrasonic horn reactor for deactivating microorganisms in water," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 36, pp. 1–13, Sep. 2012.
2. W. Q. Betancourt and J. B. Rose, "Drinking water treatment processes for removal of Cryptosporidium and Giardia.," *Vet. Parasitol.*, vol. 126, no. 1–2, pp. 219–34, Dec. 2004.
3. M. R. Doosti, R. Kargar, and M. H. Sayadi, "Water treatment using ultrasonic assistance : A review," in *International Academy of Ecology and Environmental Sciences*, 2012, vol. 2, no. 2, pp. 96–110.
4. B. Tansel, "New Technologies for Water and Wastewater Treatment: A Survey of Recent Patents," *Recent Patents Chem. Eng.*, vol. 1, no. 1, pp. 17–26, Jan. 2008.
5. N. M. Budari, M. F. Ali, K. H. Ku Hamid, K. A. Khalil, S. Zainal, and M. Musa, "Flow Cell Ultrasound Treatment : The Influence of Sonication Media and Temperature on the Disruption of Escherichia coli wild Type Cells," *Adv. Sci. Lett. J.*, p. In Press, 2017.
6. S. N. Hussain, N. de Las Heras, H. M. a Asghar, N. W. Brown, and E. P. L. Roberts, "Disinfection of water by adsorption combined with electrochemical treatment.," *Water Res.*, vol. 54, pp. 170–8, May 2014.

7. C. Yanmin, "Visible-light-driven Photocatalytic Disinfection of Bacteria by the Natural Sphalerite," The Chinese University of Hong Kong, 2011.
8. G. Andaluri, E. V Rokhina, and R. P. S. Suri, "Evaluation of relative importance of ultrasound reactor parameters for the removal of estrogen hormones in water.," *Ultrason. Sonochem.*, vol. 19, pp. 953–958, 2012.
9. O. Ayyildiz, "Ultrasonic and Air Stripping Removal of CCl4 and TCA from Water: Investigating Synergistic Effects," Illinois Institute of Technology, Chicago, 2003.
10. J. H. Gibson, D. Hai, N. Yong, R. R. Farnood, and P. Seto, "A literature review of ultrasound technology and its application in wastewater disinfection," *Water Qual. Res. J. Can.*, vol. 43, no. 1, pp. 23–35, 2008.
11. N. Gera and S. Doores, "Kinetics and mechanism of bacterial inactivation by ultrasound waves and sonoprotective effect of milk components," *J. Food Sci.*, vol. 76, no. 2, pp. 111–119, 2011.
12. M. Villamiel and P. De Jong, "Inactivation of Pseudomonas fluorescens and Streptococcus thermophilus in Trypticase Soy Broth and total bacteria in milk by continuous-flow ultrasonic treatment and conventional heating," *J. Food Eng.*, vol. 45, pp. 171–179, 2000.
13. N. M. Budari, M. F. Ali, K. H. Ku Hamid, M. Musa, K. A. Khalil, and N. F. Khairuddin, "Escherichia coli Wild Type Cells Disruption by Ultrasound Treatment for Bacterial Disinfection," in *The International Civil and Infrastructure Engineering Conference (InCIEC 2015)*, 2016, pp. 21–31.
14. S. Z. Salleh and J. S. Roberts, "Ultrasound pasteurization : The effects of temperature , soluble solids , organic acids and pH on the inactivation of Escherichia coli ATCC 25922," *Ultrason. Sonochem.*, vol. 14, pp. 323–329, 2007.
15. M. L. Moncada Reyes, "Influence of Low Sonication Intensities at Different Temperatures on Acid Tolerance, Bile Tolerance, Protease Activity, and Growth of Yogurt Culture Bacteria Lactobacillus Delbrueckii ssp. Bulgaricus and Streptococcus Salivarius ssp. Thermophilus," Louisiana State University, 2011.
16. B. Zhou, "Investigation on factors influencing ultrasound-assisted surface decontamination of fresh-cut vegetables," University of Illinois Urbana, 2010.
17. N. N. Mahamuni and Y. G. Adewuyi, "Advanced oxidation processes (AOPs) involving ultrasound for waste water treatment: a review with emphasis on cost estimation.," *Ultrason. Sonochem.*, vol. 17, no. 6, pp. 990–1003, Aug. 2010.
18. N. M. Budari, M. F. Ali, and J. Kassim, "Physical Contaminants Removal from Continuous Process of Water Filtration by a New Low-Cost Palm Shell Charcoal," *Adv. Mater. Res.*, vol. 911, pp. 397–404, Mar. 2014.
19. R. H. Jawale and P. R. Gogate, "Combined treatment approaches based on ultrasound for removal of triazophos from wastewater," *Ultrason. Sonochem.*, vol. 40, pp. 89–96, 2018.
20. M. Sivakumar and a B. Pandit, "Ultrasound enhanced degradation of Rhodamine B: optimization with power density.," *Ultrason. Sonochem.*, vol. 8, no. 3, pp. 233–240, 2001.
21. T. Yusaf and R. A. Al-Juboori, "Alternative methods of microorganism disruption for agricultural applications," *Appl. Energy*, vol. 114, pp. 909–923, Feb. 2014.
22. E. Gunerken, E. D'Hondt, M. H. . Eppink, L. Garcia-Gonzalez, K. Elst, and R. . Wijffels, "Cell disruption for microalgae biorefineries," *Biotechnol. Adv.*, vol. 33, no. 2, pp. 243–260, 2015.