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Disinfection of *Escherichia coli* Bacteria Using Combination of Ozonation and Hydrodynamic Cavitation Method with Venturi Injector

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***Abstract*— This study brings out a disinfection process of bacteria *Escherichia coli* using a combination of ozonation and hydrodynamic cavitation method. Cavitation was generated using venturi injector, and three commercial Ozonator was used for generated ozone. Various configuration method was arranged to evaluate the performance of each kind method as well as a combination of them on the disinfection process. Effect of various flow rate (3, 5, and 7 LPM) and dosage of ozone (64.83, 108.18, and 135.04 mg/h) was investigated. Combination of ozonation and hydrodynamic cavitation (7 LPM and 135.04 mg/h dosage of ozone) showed the best performance with initial concentration is 1.49 x 10^5 CFU/mL; all bacteria were disinfected for 45 min. The results are better than the single hydrodynamic cavitation method (7 LPM) which had 21% remaining of bacteria, and all bacteria were disinfected on a single ozonation method (135.04 mg/h dosage of ozone) for 60 min.**

***Keywords*— disinfection; *Escherichia coli*; ozonation; hydrodynamic cavitation; venturi injector**

I. INTRODUCTION

Jakarta has large populations that triggered people to build water supply near the septic tank. That makes around 50% of groundwater in Jakarta contaminated by *E.coli* [1]. Based on the data of Environmental Status DKI Jakarta Area 2014, Semper Barat is the most polluted area with the highest *E.coli* concentration reached 290 mpn/100mL [2]. Whereas, Peraturan Menteri Kesehatan (Regulation of Health Minister) was declared that drinking water standards must be zero from *E.coli*, less than ten mpn for piped water, and maximum 50 mpn for non-piped water.

Escherichia coli bacteria are normal flora of human digestion and animal. Some strains of *E.coli* can cause illness such as diarrhea, which is still the leading cause of death for children under five years old in Indonesia [3].

The conventional methods of disinfection of bacteria are chlorination and ozonation. The chlorination has several disadvantages including the formation of carcinogenic disinfection by-products and appearance of taste and odor problems in processed water. Although chlorination is considered relatively cheap and easy to use this disinfection method requires careful control of dosing which increases the maintenance costs [4]. The other method of disinfection process is ozonation, which is chemical oxidation technique that uses ozone as a strong oxidizing agent to disinfect bacteria. Through the process of oxidation, ozone will damage the outer wall of the cell microorganism. Disadvantages of using ozone are low solubility in water, and it is an unstable gas which quickly disappears in a few minutes in the water. So, it does not leave a residual disinfectant (residue), which causes the water to be easily re-contaminated in a short time [5]. Therefore, there is still a need for new approaches of water disinfection which could be effective, safe, and secure to perform. Combination of two or more disinfection techniques has also been applied to water treatment.

Hybrid techniques are far superior for treating water as compared to any individual physical treatment technique. [6]. Combination of ozone and hydrogen peroxide has been used for disinfection of water [7] and the combination of ozone and cavitation also used for disinfection HPC bacteria [6].

Cavitation is a phenomenon of formation, growth, and collapse of microbubbles within a liquid. Microbubbles is a bubble with a diameter less than tens microns, whereas current bubble has a diameter of several millimeters. Cavitation can increase the rate of ozone mass transfer by enlarging the surface contact area through microbubbles; cavitation can also accelerate the decomposition rate of ozone into hydroxyl radical [8]. Cavitation produces microbubbles in the water so that the gas molecules of water trapped in a bubble and will collapse and produce highly reactive free radicals such as hydroxyl radical (OH•). In hydrodynamic cavitation (HC), cavities are formed by passing the liquid through the constriction/geometry provided in line [9]. This can be generated by the orifice and venturi [6]. Hydrodynamic cavitation has been particularly useful for cell disruption. The mechanism of cavitationally induces cell disruption based on Kolmogoroff's theory of isotropic turbulence and analysis of fluid eddies created due to the collapse of the cavity. The fluid eddies smaller than the dimension of the cell will impart motions of various intensities to it, and when kinetic energy content of a cell exceeds the wall strength, the cell disintegrates [10]. The collapse of microbubbles can produce shock waves that can be the leading cause of cell disruption [11]. The effect of water disinfection on the survival of *E.coli* cell using hydrodynamic cavitation concluded that the method could kill 75% of *E.coli* bacteria and it is very promising for water disinfection [4].

Previous studies have indicated that these techniques can be inactive for a wide range of microorganisms. Furthermore, by using combination hydrodynamic and ozone, the concentration of ozone required for disinfection was reduced to half or one-third is depending upon microorganism [6] and can increase the solubility of ozone.

In this paper, we investigated the significance performance of the combination method ozonation and hydrodynamic cavitation for disinfection *E. coli* bacteria by testing the performance of each ozonation method, hydrodynamic cavitation with venturi injector and a combination of both.

II. MATERIAL AND METHOD

A. Materials Preparation

Synthetic wastewater contaminated by *E.coli* culture was used for the experimental investigation. The test water 5 L was prepared by diluting a pure culture of *E.coli* 50 mL with a concentration of 10^8 CFU/mL. The concentration of *E.coli* was calculated before and after disinfection. In this study water that has been contaminated by *E. coli* bacteria with initial concentrations ranging from 10^5 CFU/mL. Potassium permanganate ($KMnO_4$ p.a) from Merck was used in the hydroxyl radical quantification. Indigo reagent (HACH Cat. 2518025) to determine the residual ozone concentration. Commercial Ozonator generated ozone. There are three ozonator used for dosage ozone variation, two from X-troy and one from HANACO.

B. Instrumentation

The experimental setup is shown in Fig. 1. The setup includes a feed tank, centrifugal pump (Sanyo PH236A 400 W), ozone generator (Ozonator), injector (venturi Mazzei type 384), thermo circulator, flow meter, and pressure gauge. In the tank is added a cooling coil was connected to the thermal circulator to cool the feed water. Cooling is needed to ensure that the disinfection process in this study is not caused by temperature rise in the use of any method. For single ozonation method, the water jet injector is replaced with a pipe. For single hydrodynamic cavitation method, the ozonator is not used. Colorimeter DR-890 to analyze residual ozone.

C. Instrumentation

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D. Procedure

1) Quantification of Hydroxyl Radicals: The experiment used water without *E.coli* bacteria. Aquademin (5 L) has been carried out using three method configuration, which are single ozonation, single hydrodynamic cavitation, and the combination of both for 60 min and samples were taken from the reservoir tank at a fixed interval of time for further analysis. The experiment using hydrodynamic cavitation was conducted at a various flow rate (3, 5, 7 LPM). The experiment using ozonation method was conducted at various dosage of ozone (64.83, 108.18, 135.04 mg/h) with 7 LPM of flow rate The experiment using combination of ozonation and hydrodynamic cavitation was conducted at various initial dosage of ozone (64.83, 108.18, 135.04 mg/h) with 7 LPM of flow rate. The samples were analyzed by permanganometric titration to determine the number of hydroxyl radicals generated.

2) Disinfection of Escherichia coli: Disinfection of *Escherichia coli* has been carried out using single ozonation, single hydrodynamic cavitation, and a combination of both. For all of the schemes used 7 LPM of flow rate. Ozonation and the combination were also conducted at a various dosage of ozone (64.83, 108.18, 135.04 mg/h). All the experiments were performed for 60 min and every 15 min; sample were taken from sampling ports for analysis of residual ozone concentration and bacteria concentration. Experimental data of bacteria concentration were measured by TPC (total plate count) method.

III. RESULT AND DISCUSSION

A. Quantification of Hydroxyl Radicals

In addition to ozone, hydroxyl radical also has a vital role in the disinfection of *Escherichia coli*. Therefore, before the experiment of disinfection using synthetic wastewater, quantification of hydroxyl radical for each of configuration method was investigated. To study the effect of each configuration method and the variety of the operational condition against the amount of generated hydroxyl radical. When hydroxyl radical formed, it would react non-selectively with other compounds around it [5]. Therefore, the quantification of hydroxyl radicals cannot be done directly, but by using an approach which is permanganometric titration method.

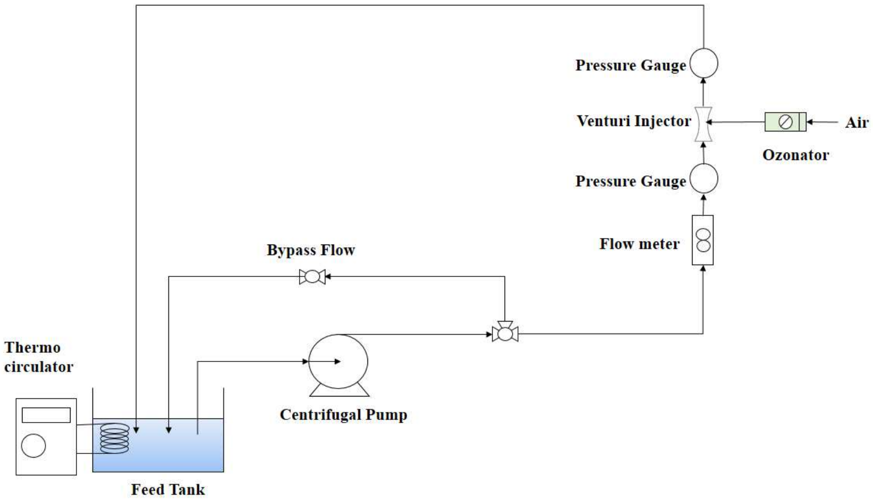

Fig. 1 Experimental setup of the combined method of ozonation and hydrodynamic cavitation

Permanganometric is an oxidation-reduction titration method that used potassium permanganate ($KMnO_4$) as a titrant and oxidizing agents. $KMnO_4$ as a titrant worked by oxidizing the reducing agent in the solution until the end of the titration reached. In the aquademin, no other compound can be oxidized, so the formed of hydroxyl radicals does not react with other compound but with the hydroxyl radical itself. Then would form hydrogen peroxide (H_2O_2) which will be the approximation in the quantification of hydroxyl radical.

1) Quantification of Hydroxyl Radical in Hydrodynamic Cavitation Method: Fig. 2 shows that the amount of hydroxyl radicals increases with an increase of flow rate. The various flowrate (3, 5, 7 LPM) yielded 4.93, 5.185, and 5.53 mg/L hydroxyl radical, respectively. This results can be attributed to the fact that the fluid velocity in the tubule will be higher than the inlet. Increasing the inlet velocity will increase the pressure drop at the tubule which is causing the cavitation effect to be higher, and lead to increase the amount of hydroxyl radical [12]. This results can be related to the cavitation number. It is defined as the ratio of pressure drop between the throat and extreme downstream section of the cavitating device to the kinetic head at the throat [13].

Some cavities are generated under ideal condition ($C_V \leq 1$), but in many cases, cavities can get generate at $C_V > 1$ due to the existence of little-dissolved gases and suspended particles [14]. In this experiment, the generated cavitation number at 3, 5, and 7 LPM were 0.65, 0.59, and 0.54, respectively. So, as the flow rate increases, cavitation number decreases which in turn generates more hydroxyl radicals. In the (HC) method hydroxyl radicals formed by thermal dissociation reaction of water vapor and oxygen occurring in microbubbles as shown in equation (1) [8].

$$H_2O \rightarrow \bullet OH + H^{\bullet} \quad (1)$$

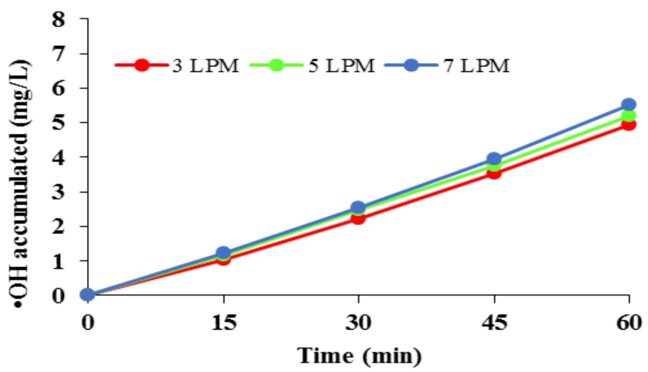

Fig. 2 Effect of flow rate on hydroxyl radical production by single hydrodynamic cavitation

2) Quantification of Hydroxyl Radical in Ozonation Method: The evaluation of hydroxyl radical by ozonation method was conducted with a various dosage of ozone. Increasing the dosage of ozone will increase the amount of hydroxyl radical shown in Fig. 3. For various dosage of ozone (64.83, 108.18, 135.04 mg/h), yielded 5.44, 6.59, 6.76 mg/L hydroxyl radical, respectively. Residual ozone at a dosage of ozone 135.04 mg/h was measured. The residual ozone increased as time goes by is because due to the absence of bacteria that can be disinfected. In single ozonation method (O), production of hydroxyl radicals was formed by ozone decomposition. Ozone will decompose to produce oxygen and oxygen atoms because of its unstable in water. The oxygen atoms will react with water to form hydroxyl radicals as shown in equation (2) and (3):

$$O_3 \rightarrow O_2 + O\bullet \quad (2)$$

$$O\bullet + H_2O \rightarrow 2\bullet OH \quad (3)$$

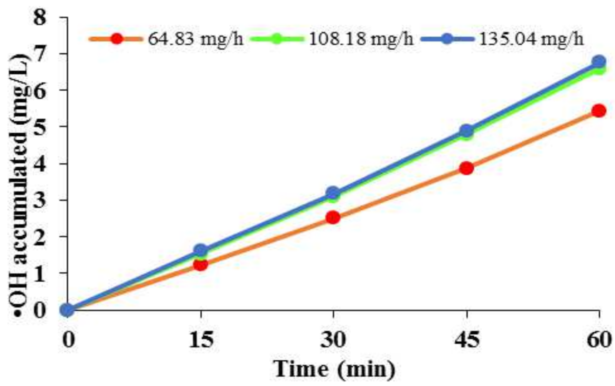

Fig. 3 Effect of flow rate on hydroxyl radical production by single ozonation

3) Quantification of Hydroxyl Radical in Combined Method: The combination of ozonation and hydrodynamic cavitation also conducted with a various dosage of ozone. The amount of hydroxyl radicals production using a combination of that method was greater than the single ozonation or hydrodynamic cavitation. The number of hydroxyl radicals of various dosage of ozone (64.83, 108.18, 135.04 mg/h) shown in Fig. 4 and the results is 7.23, 6.93, 5.70 mg/L, respectively. The results are also higher than the ozonation or hydrodynamic cavitation method. The results indicate the increasing of ozone mass transfer due to the mechanical effect of hydrodynamic cavitation, and the addition of hydroxyl radical from hydrodynamic cavitation itself as a chemical effect. Residual ozone in this mixed method with a dosage of ozone 135.04 mg/h also measured.

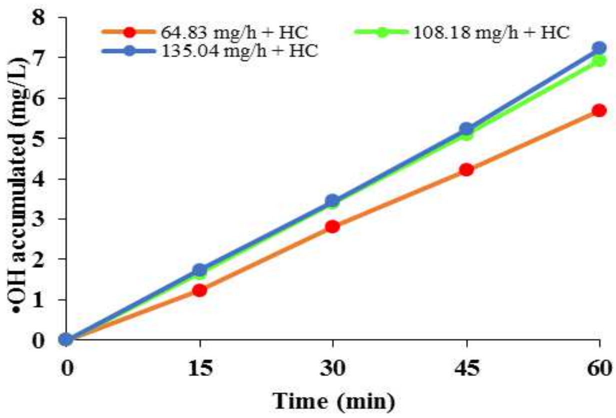

Fig. 4 Effect of flow rate on hydroxyl radical production by the combined method

B. Disinfection of Escherichia coli

Percentage of C/C_0 was investigated to evaluate the performance of disinfection method. C_0 is the initial concentration of bacteria and C is the concentration at (t) time. The percentage C/C_0 shows the percentage of the remaining amount of *Escherichia coli* bacteria in the feed. As mentioned earlier, the disinfection of *Escherichia coli* was conducted with various configuration method.

1) Circulation Test: circulation test is a disinfection process without ozone splitting and installed venturi injector. This is important because we must ensure that the disinfection process in this study occurs because of ozone and hydrodynamic cavitation, not due to collisions on pipe fittings and impeller on the pump. The test was used 7 LPM of flow rate with 60 min disinfection process. There are two variations; used cooling and without cooling.

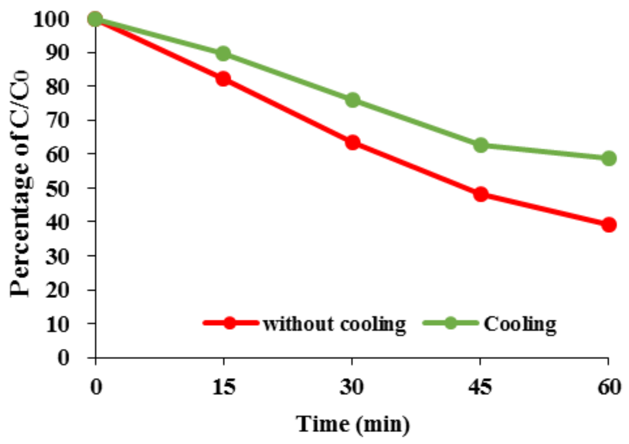

Fig. 5 Percentage of C/C_0 in circulation test (7 LPM)

In Fig. 5, show that the remaining bacteria was higher in the circulation test with the cooling. This indicates that %-disinfection without cooling is more prominent than with cooling. From the results of circulation test, we get the result there is decreased of bacteria concentrations. This suggested that disinfection may occur even if the final concentration of bacteria was still high.

TABLE I
SUMMARY OF CIRCULATION TEST WITH AND WITHOUT COOLING

Time (min)	Cooling		Without cooling	
	Tempe-rature (°C)	C (CFU/mL)	Temperature (°C)	C (CFU/mL)
0	27	1.82E+05	27.6	2.03E+05
15	31.8	1.63E+05	36	1.67E+05
30	34	1.38E+05	43	1.29E+05
45	36	1.14E+05	47.8	9.80E+04
60	37	1.07E+05	51	7.98E+04

Disinfection in circulation test occurs due to the turbulence formed during the circulation process and collisions between the flows down from a pipe in contact with water in feed tank cause the collapse of bubbles. During the process, there is a temperature increase as shown in Table 1 cause by continuous impeller rotation resulting friction between water and pump impeller. Also, temperature increase also caused by the fluid friction on the pipe wall continuously which result in heat. Temperature control was required during disinfection process to ensure that the disinfection process was not caused by temperature rise in the use of any method. Therefore, in the disinfection process was used cooling that connected from coil to compressor.

2) Disinfection in Hydrodynamic Cavitation Method: In single hydrodynamic cavitation method with venturi injector showed a result that there are still about 21% *E.coli* bacteria in feed water with concentration 4.57 x 10^4 CFU/mL from initial concentration 2.15 x 10^5 CFU/mL as can be seen in

Fig. 6. Disinfection of *E.coli* using hydrodynamic cavitation can occur due to mechanical, chemical, and thermal effects.

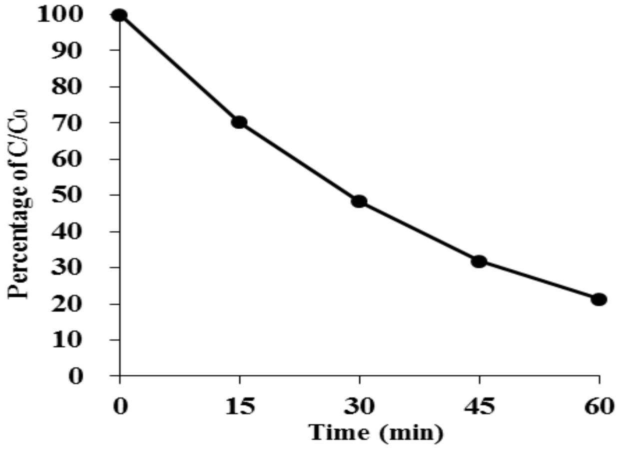

Fig. 6 Percentage of C/C_0 in hydrodynamic cavitation method (7 LPM)

In mechanical effect, there are several possibilities. Disinfection in hydrodynamic cavitation occurs due to turbulence and the formation of eddy flow. High speed made the flow turbulence bigger. The flow of eddy formed was smaller than the physiological of the cell so that it will provide movement from any directions, and when the kinetic energy in the cell exceeds the strength of cell wall, the cell would have destroyed [15]. Disinfection of the hydrodynamic cavitation method caused by the formation of the shock wave in the flow, the difference of pressure caused microbubbles to collapse. The collapse of microbubbles can cause cell disruption.

Based on chemical effects, disinfection with hydrodynamic cavitation method can occur due to the formation of free radicals from the collapse of bubbles. During microbubbles dissolved in water, gas molecules from the water trapped inside bubbles would encounter dissociation reactions.

Thermal effects occur from local hotspot formation which gives very high local temperature and pressure. These conditions caused by the collapse of bubbles that generated from cavitation. The high temperature can destroy bacteria.

3) Disinfection in Ozonation Method: In single ozonation method, best result was reached using 135.04 mg/h dosage of ozone with the initial concentration is 1.32 x 10^5 CFU/mL and during 60 min all bacteria were disinfected. Using 108.18 mg/h dosage of ozone, with initial concentrations of 1.49 x 10^5 CFU/mL are decreased to 5.63 x 10^3 CFU/mL, there are still 4% bacteria in the feed water. While using 64.83 mg/h dosage of ozone, the concentration decreased from the initial concentration of 2.16 x 10^5 CFU/mL to 2.37 x 10^4 CFU/mL; there are still about 11% of *E.coli* bacteria in the feed water. The results obtained that greater used dosage of ozone, remaining bacteria in the feed water will be less because of the number of oxidants that destroy the wall of cell bacterial more formed as can be seen in Fig. 7.

The more production of ozone using Ozonator will generate the more powerful oxidizing agents that will destroy cell wall. Moreover, soluble ozone in water will produce hydroxyl radicals that have bigger oxidation potential. With larger oxidation potential, hydroxyl radical help to accelerate the destruction of *E.coli* by binding with cell wall components.

Furthermore, ozone inactivates bacteria by through oxidation reactions. The cell membrane under attack in the first time and after that ozone attacks glycoproteins, glycolipids, or specific amino acids, and acts upon the sulfhydryl groups of specific enzymes. With a continuous process, the bacterial cell begins to break down after being in contact with ozone and the cell membrane is perforated during this process and cell disintegrates or suffers cellular lysis ([16], [17]).

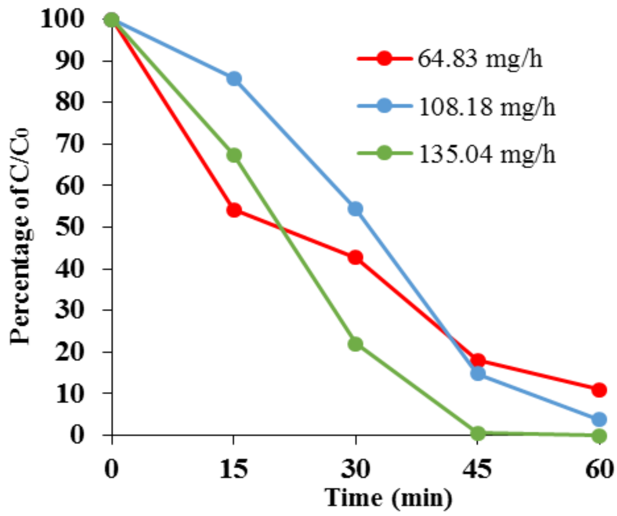

Fig. 7 Percentage of C/C_0 in ozonation method (7 LPM)

4) Disinfection in Combination Method: In combination method, ozone was injected directly into the venturi injector and then circulated to the feed tank and continuously. The purpose of combining the ozonation and hydrodynamic cavitation method is to know the significance of performance in disinfection process. This combination aims to increase ozone solubility in water by increasing contact area.

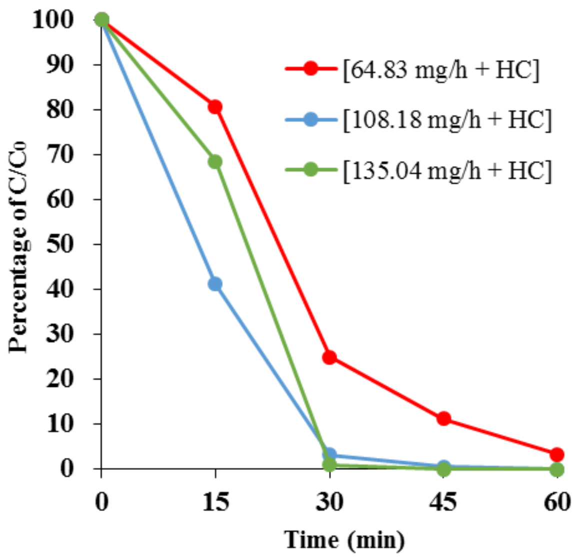

Fig. 8 Percentage of C/C_0 in combination method (7 LPM)

TABLE III SUMMARY OF DISINFECTION PROCESS OF ESCHERICHIA COLI BACTERIA

Variation	Initial Concentration (CFU/mL)	Final Concentration (CFU/mL)	Percentage of C/C_0
7 LPM*	2.15E+05	4.57E+04	21%
64.83 mg/h	2.16E+05	2.37E+04	11%
108.18 mg/h	1.49E+05	5.63E+03	4%
135.04 mg/h	1.32E+05	0 (60 min)	0
64.83 mg/h + HC	2.07E+05	6.79E+03	3%
108.8 mg/h + HC	1.89E+05	0 (60 min)	0
135.04 mg/h + HC	1.49E+05	0 (45 min)	0

*best performance in HC methods

The result as shown in Fig. 8, using 64.83 mg/h+HC dosage of ozone with initial concentration of 2.07 x 10^5 CFU/mL are decrease to 6.79 x 10^3 CFU/mL and using 108.18 mg/h+HC, with initial concentration of 1.89 x 10^5 CFU/mL are decreased to 0 during 60 min disinfection process. So, the disinfection process reached 100%. Best results showed in combination method using 135 mg/h+HC dosage of ozone; the disinfection reached 100% at 45 min with initial concentration 1.49 x 10^5 CFU/mL.

The performance of disinfection process can be assessed by using two criteria which are a percentage of C/C_0 and concentration. To simplify the result in this experiment, we can see Table 2. It is shown that the combination ozonation and hydrodynamic cavitation showed the best result. It is proven that hybrid techniques are far superior for disinfection *E.coli* bacteria.

In disinfection process, we also measured residual ozone. Residual ozone at a various dosage of ozone was measured. Resulted showed residual ozone decreased as time goes by is because ozone used to disinfect bacteria and occurs decomposition of ozone

C. Ozone Residual in Disinfection Process

Ozone residual concentration represented the amount of ozone in a solution that does not react with the bacteria. The result of ozone residual on each disinfection process is shown in Fig. 9. As shown below that ozone residual some have increased, but others decreased. Increasing the amount of residual ozone in the solution can be caused by the reduced concentration of bacteria to be disinfected so that the ozone required to disinfect the amount slowly decreases. Moreover, for the decreased, indicate that increasing contact time ozone was used and reacted to disinfect bacteria so that the amount will decrease or decomposed into hydroxyl radical which can be used to disinfect *E.coli* bacteria

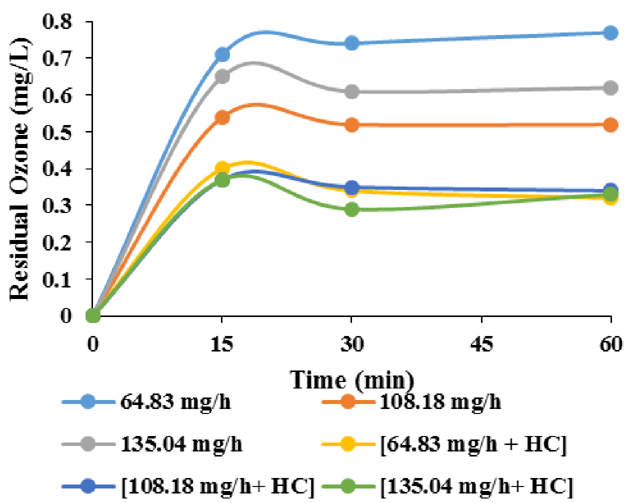

Fig. 9 Profile of residual ozone concentration in Disinfection Process

IV. CONCLUSIONS

From the studies carried out in this work, it can be observed that combination of ozonation and hydrodynamic method are better for disinfection bacteria compared with single ozonation or single hydrodynamic cavitation. Combination of ozonation and hydrodynamic cavitation (7 LPM and 135.04 mg/h) dosage of ozone showed the best performance with the percentage of C/C_0 0% for 45 min. This result is better than the 7 LPM hydrodynamic cavitation method, and 135.04 mg/h dosage of ozone at ozonation method separately, which are has a percentage of C/C_0 21% and 0% for 60 min accordingly.

The disinfection of *Escherichia coli* is affected by the dosage of ozone. The higher the ozone dosage, the percentage of C/C_0 will be smaller, in other words, the performance of disinfection will get better.

NOMENCLATURE

mpn most probable number
LPM liter/min
CFU/mL colony forming unit/mL

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