Development of ozone sterilization system based microcontroller for *E. Coli* bacteria sterilization

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**Abstract.** It has been created microcontroller-based ozone sterilization system which is used for *E. Coli* bacteria sterilization. The created system consists of a neon sign transformer, corona electrode, ozone gas chamber container and ozone gas metering systems. Neon sign transformer and corona electrode are then assembled into an ozone generator that works on the principle of corona discharge. The use of ozone generators is controlled by microcontroller system. Ozone gas is produced by ozone generators which housed in a chamber and equipped by ozone gas indicator. The results of ozone gas effect testing in *E. Coli* with varied exposure of 15, 30 and 45 minutes showed the decrease number of *E. Coli* colonies of tested bacteria samples compared to the control sample without ozone gas exposure.

**1. Introduction**

In medical front, preventing hospital infection or pathogenic disease, for instance surgical instrument, sheet, food, and human body, has to be sterilized. These things cannot be applied in unitary sterilization technology. Therefore, the different sterilization methods are chosen depending on targets. For example, to sterilize small-sized and heat-resistant instrument, the autoclave apparatus, which utilizes heat, is used. However, this method cannot be applied to medical equipment which made of plastic or latex because their behaviour is destroyed by the heat. One of the applied methods for the cases is the plasma sterilization method [1]. Ozone is a kind of plasma sources used for sterilizing.

Ozone gas is the gas occurred from 3 atoms of oxygen gathering. The oxygen gas can burst to be oxygens free atoms (O). In addition, they are gathered in O₂ into ozone gas O₃ [2]. Ozone is a practically transparent gas with very strong absorption in the region of a 245 nm wavelength. Besides, ozone is considered as a powerful disinfecting and oxidizing agent. Due to the reasons, it is used in a wide range of applications such as wastewater treatment, food processing, fire restoration, hospital disinfectant, etc. When ozone contacted with organic compounds or bacteria, the extra atom of oxygen will destroy the contaminant by oxidation. Furthermore, ozone will neutralize virtually all organic odors, specifically those containing carbons as their base element [3]. Ozone gas usually generated from corona discharge that arise from the specific electrode applied by a high voltage power supply [4].

Standard test methods typically include indicator test organism which represent of those found in surface to be sanitized. *E. Coli* is the most frequently used principal test organism for sanitizer studies. It is complemented by the selected secondary organism for the specific use recommended by sanitizer. For instance, *E. Coli* and *Staphylococcus aureus* are used for testing the same food-contact surface sanitizer; *E. Coli* and *Micrococcus caseolyticus* are often used for dairy sanitizers, and etc [5]. The
usage of sanitizer for *E. Coli* sterilization from previous research was done using ultraviolet light [6-7], Dielectric Barrier Discharge (DBD) air plasma [8], helium plasma and ozone gas [9]. This study developed ozone sterilizer based microcontroller and investigates the effect of ozone gas to the *E. Coli*.

2. Materials and methods

2.1. Ozone generator and microcontroller system

In this study, ozone generator system consisting neon sign transformer and corona electrode is made to produce ozone gas which used as sterilization process. Neon sign transformer serves as a high voltage source connected to the corona electrode. In addition, it results corona discharge in the electrodes and produce ozone gas. Corona electrode is made from two cylindrical electrodes with a glass as dielectric barrier between them. Corona electrode’s picture is shown in Figure 1.

![Corona electrode](image)

**Figure 1.** Corona electrode

Microcontroller-based controller system is developed to aid the use of ozone generator. Through this system, the use of ozone generator and ozone gas detector can be performed easily. Some components required to actualize the controller system, for instance microcontroller module arduino, MQ131 sensor, LCD 16x2, memory card module, Real Time Clock (RTC) module, and a rotary switch. The circuit of microcontroller system is presented in Figure 2.

![The circuit of microcontroller system](image)

**Figure 2.** The circuit of microcontroller system

The algorithm is used for the microcontroller system to determine the time setting of ozone generator with the rotary switch considered as the first step. When the start button is pressed, the
ozone generator will turn on according to the specified time setting and start to read the sensor data which automatically stored to the memory card. When the ozone generator stops working, the ozone detection continues to run until the reset button is pressed. The flow chart or flow diagram program from proposed algorithm is shown in Figure 3.

![Flow chart algorithm of the microcontroller system](image)

**Figure 3.** Flow chart algorithm of the microcontroller system

Generators electrode ozone and ozone sensors are placed in the chamber so that the ozone gas that will be used for sterilization accommodated in the chamber. Before the ozone generator is used, the pressure inside the chamber is set smaller than the outside air pressure. As the result, if the ozone gas generated by the ozone generator, there will be no ozone gas coming out during the use of ozone gas in the sterilization process.

2.2. **Testing on the E. Coli bacteria**
The survival rate of *E. Coli* was measured after ozone exposure under time variation to investigate the sterilizing effect of ozone gas. The cultured *E. Coli* bacteria on agar plates was used as indicator bacterium. During the sterilization treatment, the agar plate was placed in the chamber. After ozone treatment, colony condition of agar plate was observed for 24 hours in incubation.

3. **Results and discussion**
The prototype of microcontroller system to control the ozone generator was developed in this study as it is represented in Figure 4. In the LCD display output voltage, the sensor is shown as an indicator of the detection presence or absence of ozone gas. Automatically, if there is no detected ozone gas, the output voltage of the voltage sensor indicates nearly 5 volts (4.95 volts). Conversely, if the ozone gas is detected, the sensor output voltage will be decrease (approaching to zero volts). After the start button is pressed, the ozone generator will start to switch on and the valve opened, so that oxygen
comes into the chamber and the process of ozone gas monitor begin to operate. Ozone sensor output voltage data will be stored automatically on the memory card in every second. The monitoring process of ozone gas will stop when the reset button is pressed. The test results showed the control system has worked properly in accordance with the created algorithm in the microcontroller program.

Figure 4. Prototype of microcontroller system

The concentration of ozone gas produced by ozone generator was determined by iodometric titration method [10]. There were several stages in iodometric titration as follows:

1. Prepared the required chemicals: 0.15 M, KI; 0.1 M, H$_2$SO$_4$; Na$_2$S$_2$O$_3$, 0.035 N and 1% starch solution.
2. Turned on the ozone generator for 30 seconds with oxygen flow rate of 1 litre (L) / minute, so that the formed ozone gas is accommodated in the chamber.
3. The ozone gas flow to a KI solution were placed in 20 ml Erlenmeyer. Furthermore, a KI solution will change into yellow indicating the reaction of ozone gas against a KI solution. In the experiment, discoloration occurred when ozone flow the volume of 240 ml.
4. dissolved the 4 ml of H$_2$SO$_4$ solution and 3 drops of starch to KI, so that KI solution turns into a deep blue colour.
5. Titrated with a Na$_2$S$_2$O$_3$ solution. The titration process was stopped when KI solution turned into clear and recorded the titrated volume of Na$_2$S$_2$O$_3$ solution. Mass of ozone trapped in KI can be found in this equation (1)[10].

$$mass\ of\ O_3 = 24 \times V_t \times N_t$$  \hspace{1cm} (1)

where :  
24 = Conversion factor = 24000 me/L per 1000ml/L
$V_t$ = Volume of sodium thiosulfate, in mL
$N_t$ = Normality of sodium thiosulfate, in mg/me

Ozone concentration can be determined using equation:

$$Concentration\ of\ O_3 = \frac{mass\ of\ O_3}{Volume\ of\ O_3}$$  \hspace{1cm} (2)

From the experiment, the obtained concentration of O$_3$ = 0.84 mg/L.

The experiment results of E. Coli bacteria that have been exposed to ozone is figured in Table 1. Table 1 shows the decrease number of E. Coli bacteria on the medium exposed by ozone with three variations of exposure time (15, 30 and 45 minutes) compared to media without ozone exposure (control sample). When it was exposed by ozone for 15 minutes, the number of colonies was $6.7 \times 10^6$ CFU/ml. In addition, the colonies number were $5.3 \times 10^6$ CFU/ml with 30 minutes exposure and $3.4 \times 10^6$ CFU/ml with 45 minutes exposure. Meanwhile, bacteria media that was not exposed to ozone reached $8.1 \times 10^6$ CFU/ml of the number of colonies. The longer time of exposure influenced the amount of bacterial growth. It revealed the relation between ozone gas exposure time and the growth of the E. Coli bacteria. From the results, it was found out that ozone gas can be used for the bacteria sterilization, especially E. Coli.
Table 1. The number of *E. Coli* colonies condition after exposure and control sample without ozone exposure

| No | Time of exposure | Colonies Number |
|----|------------------|-----------------|
| 1  | 15 minutes       | $6.7 \times 10^6$ CFU/ml |
| 2  | 30 minutes       | $5.3 \times 10^6$ CFU/ml |
| 3  | 45 minutes       | $3.4 \times 10^6$ CFU/ml |
|    | Control sample   | $8.1 \times 10^6$ CFU/ml |

4. Conclusion

A microcontroller-based sterilization system for *E. Coli* bacteria sterilization which created ozone generator and control system with ozone gas indicator has been developed successfully. The test results showed that the control system has worked properly in accordance with an algorithm created in the microcontroller program. Thus, the *E. Coli* bacteria test results indicated the decrease bacteria number the number on the medium of *E. Coli* bacteria with ozone exposure with three variations of exposure time (15, 30, 45 minutes) compared to bacteria media without ozone exposure (control sample). Based on the variations of exposure time, it is shown that the longer exposure time leads a little amount of bacterial growth.

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