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Development of the Sterilization Box for Medical Equipment with an Ozone Gas Leak Sensor Feature

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Abstract. One of the efforts to control the growth of microorganisms is sterilization. The sterilization process can use ozone gas, which is a triatomic form of the element oxygen. Ozone acts as an oxidizing agent capable of destroying the structure of bacterial cell walls, and their molecules are unstable and easily decomposed into oxygen, so that ozone can be applied in sterilization technology for medical devices. In this work, a sterilization box with an ozone generator has been designed with a MQ-131 ozone sensor as an indicator if there is a leak in the box. The output voltage of the ozone generator is 4 kV with a current of 30 mA. We developed an instrument using an Arduino nano as a microcontroller for reading sensor values and displaying sensor values on an LCD monitor. For testing the sterilization effect of the ozone box, a *Staphylococcus aureus* bacterial sample was used. For the sterilization object, we designated stethoscopes and thermometers as medical equipment. The optimum time for sterilizing medical devices on the sterilization ozone box was 20 minutes, which can reduce the colony of *S. aureus* bacteria with an ozone concentration of 4.94 ppm.

Keywords: Leak sensor; Medical equipment; Microcontroller; Ozone; Staphylococcus bacteria; Sterilization

1. Introduction

Ozone (O_3) is a strong oxidizing agent and a triatomic compound of the element oxygen (O). Ozone is also an unstable compound that quickly decomposes at ambient temperature (Ebbing & Gammon, 2017). The sterilization process using ozone occurs through a direct oxidation process. Ozone oxidation has the ability to destroy cell membranes and the outer walls of microorganism cells, leading to cell dead (Takayama et al., 2006). Ozone is often used as a sterilization method because it is oxidative and easy to decompose, among others, to eliminate bacteria in the soil/soil sterilization. It is applied in the agricultural field because ozone can kill bacteria, viruses, and fungi in the soil as well as in irrigation systems, and then ozone will decompose into oxygen (O_2) (Suryawan et al., 2021; Munarso et al.,

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2020; Pawłat et al., 2010).

Ozone production can be carried out in various ways, such as through electrochemical reactions, UV irradiation methods, and plasma technology. Plasma is the most abundant of these various methods (Suksri et al., 2009). If the temperature continues to be raised, the atoms will decompose into charged particles that move freely (electrons and positive ions) and enter the plasma state (Bellan, 2008). Plasma also be used as a sterilization method for medical devices using Plasma Activated Water (PAW) with some modifications to its physicochemical properties, such as pH, temperature, ORP, and nitrite concentration (Abuzairi et al., 2018). Moreover, UVC was employed for room sterilization and disinfection (Rusdinar et al., 2021). The authors claim that the UVC can reduce and kill airborne bacteria; therefore, it can disinfect different types of rooms (e.g., isolation, operating, and public rooms) contaminated with hazardous bacteria, such as COVID-19 (Rusdinar et al., 2021; Berawi, 2021).

Recently, ozone has also been applied in some hospitals as a method for sterilizing medical equipment and other equipment in hospitals because it has been proven effective against vegetative bacteria, fungi, mycobacteria, and bacterial spores (Dufresne et al., 2004). In the food sector, ozone is produced in dielectric barrier discharge plasma (DBDP) and then applied to maintain the quality of amino acids in fish (Sosiawati *et al.*, 2014). In addition, ozone could be a hand sanitizer using air and tap water enriched by hydrogen peroxide (El Shaer et al., 2017). Ozone can be used to combat coronaviruses both inside and outside the body (Manjunath et al., 2021).

Ozone is very useful for sterilization; however, at high concentrations, it is also harmful to humans. From previous studies on the design of sterilization instrumentation using ozone gas, no study has developed sterilization instrumentation with an ozone gas leak sensor. Therefore, in this study, we developed a sterilization box for medical equipment with an ozone gas leak sensor feature.

2. Methods

2.1. Ozone sterilization box

The sterilization box is shown in Figure 1. The ozone generator uses a Dielectric Barrier Discharge (DBD) configuration. The ozone generator will be put in a sterilization box, which functions as a safety so that ozone is not inhaled or contaminates other objects. The ozone generator and samples will be put into the storage box, and then a hole will be made as an outlet for the cable. This ozone sterilization box is also connected to a timer relay and switch to make it easier for users to set the duration of the ozone generator's activation.

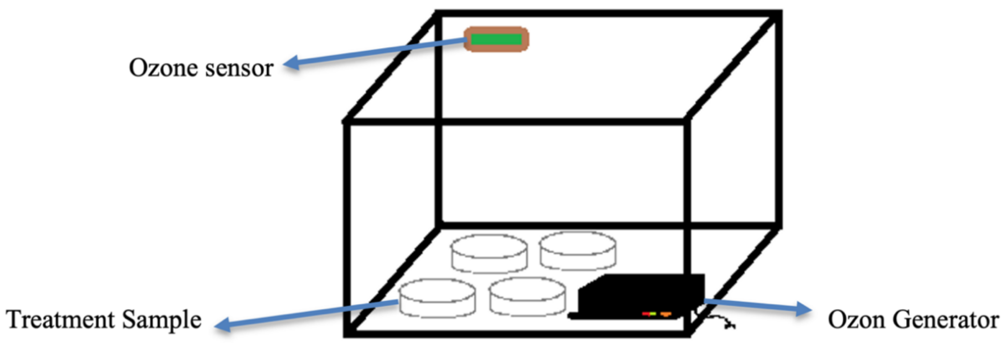


Figure 1 Sterilization box with ozone gas leak sensor feature

2.2. Ozone gas leak sensor circuit

The ozone sensor MQ-131 is connected to the Arduino nano, as shown in the schematic in Figure 2. The sensitive material of the MQ-131 ozone sensor is a metallic oxide semiconductor, which has high conductivity in clean air. When ozone gas is present, the sensor's conductivity decreases as the gas concentration increases. Aside from sensitivity to specific ozone, the MQ-131 sensor also has a sensitivity to strong oxides, Cl_2 and NO_2. In order to work optimally, it requires preheating for approximately 48 hours. The MQ-131 sensor can be applied as a domestic or industrial ozone concentration alarm and can also be used as a portable ozone concentration detector. The MQ-131 ozone sensor has a long life, easy to apply and is flexible enough to be connected to a microcontroller for various applications.

The MQ-131 ozone sensor circuit is added outside the sterilization box and then connected to the LCD to be able to monitor the level of ozone that comes out/leaks during the treatment process. This is done as a safety system when a leak occurs or the box is not tightly closed. This sensor was also used in other Covid-19 research for controlling ozone concentration (Dave et al., 2020).

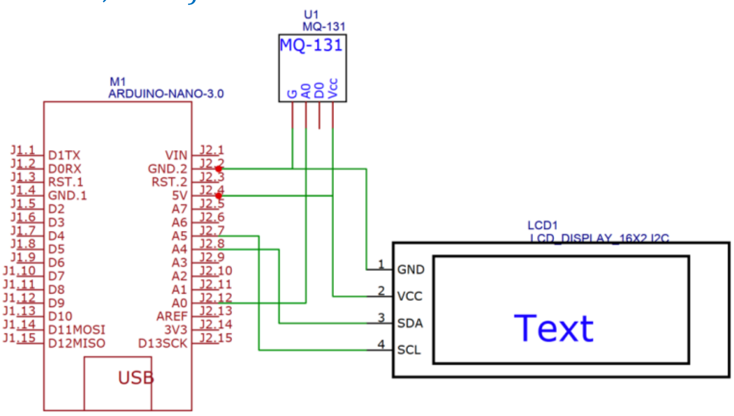


Figure 2 Schematic of ozone gas leak sensor circuit

2.3. Ozone concentration measurement

A plasma generator is connected to a high voltage generator at the DBD to see the glow discharge and ozone produced. Ozone concentration is calculated quantitatively with an air quality monitor the Zuidid DM502-O3 where there is one feature that can measure ozone concentration in ppm units.

2.4. Validation of ozone sterilization

In this study, we used a *Staphylococcus aureus* bacterial sample to validate the sterilization effectiveness of ozone. *S. aureus* is a Gram-positive bacterium in the form of a coccus. When the ozone generator circuit has been designed, the next step is to test the effectiveness against staphylococcus bacteria on medical equipment, which is stethoscopes and thermometer guns (Abuzairi et al., 2021). Bacterial samples were taken from a stethoscope and thermometer, which were then swabbed and planted in Mannitol Salt Agar (MSA) and Plate Count Agar (PCA) media. Samples that have been planted are then treated using an ozone generator, which is designed with a treatment duration of 5 minutes, 10 minutes, and 20 minutes. Samples that have been treated will then be cultured for two days, and after counting the bacterial colonies formed, they will be counted and analyzed. In the treatment method, the device is put into a sterilization box to be treated according to the optimal time obtained in the first experiment, then cultured in the media for 2 days, and the bacterial colonies that form will be counted and analyzed. Figure 3 shows the stages of bacterial sterilization.

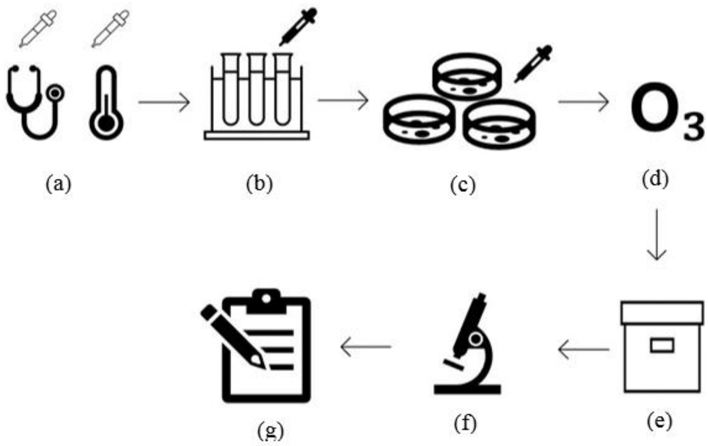


Figure 3 Stages of bacterial sterilization with an ozone sterilization box. (a)-(b) Medical equipment is swabbed, and the sample is stored in a transport medium. (c) The sample is planted on a PCA/MSA plate. (d) the sample-containing plate is treated with ozone. (e) The plate is incubated at 36°C for 48 hours. (f)-(g) Colony counting and cell observation under the microscope

3. Results and Discussion

3.1. Ozone generator

Figure 4(a) shows the device before it is electrified, and Figure 4(b) shows it after it is electrified. When electrified, a glow discharge appears on the dielectric barrier, indicating a plasma discharge process. This is in accordance with the parameters for the ozone generator, which are indicated by the presence of a glow discharge when electrified. In addition, there is a distinctive smell of ozone. Based on the technical specifications in the circuit and the simulation results, the output voltage is 4 kV with a current of 30 mA. This circuit will be measured ozone concentration and designed for a sterilization box.

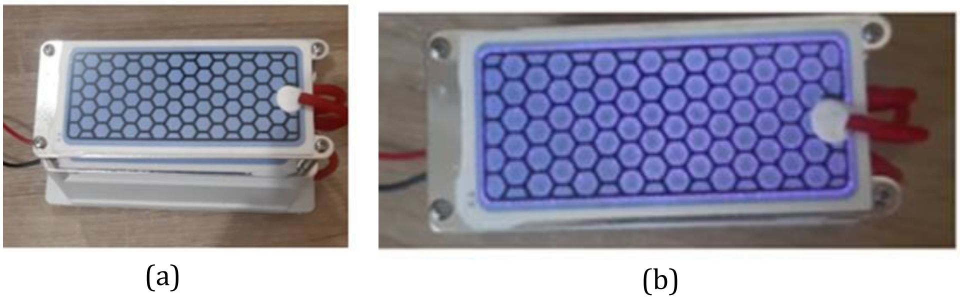


Figure 4 (a) Ozone generator not electrified. (b) Ozone generator electrified

3.2. Ozone sensor

The ozone gas leak sensor circuit is attached to the upper of the sterilization box, as shown in Figure 5(a). The MQ-131 sensor could detect the ozone concentration up to 1000 ppm, with a sensitivity (ozone/air) is 2 in 50 ppm ozone (Zhengzhou Winsen Electronics Technology, 2021). The side of the storage is perforated for cable output, which will be connected to switches and timers to make it easier to turn off and turn on the device because it would be better if during the treatment process, there were no people in the treatment room and there is air circulation in the room to anticipate if something happened like an ozone gas leak. On the outside of the front sterilization box, a series of sensors MQ-131 are provided with an LCD Figure 5(b) to monitor during treatment if there is ozone leaking from inside the box, as shown in Figure 5(c). This is added because, according to WHO regulations and some countries limits, the ozone concentration in a room should not be more than 0.1 ppm. Human exposure to ozone is primarily by inhalation, but reactions on the skin are also reported (Salonen et al., 2018). Acute and chronic health effects and the contributions of ozone to morbidity and mortality are summarized in WHO documents (WHO, 2021).

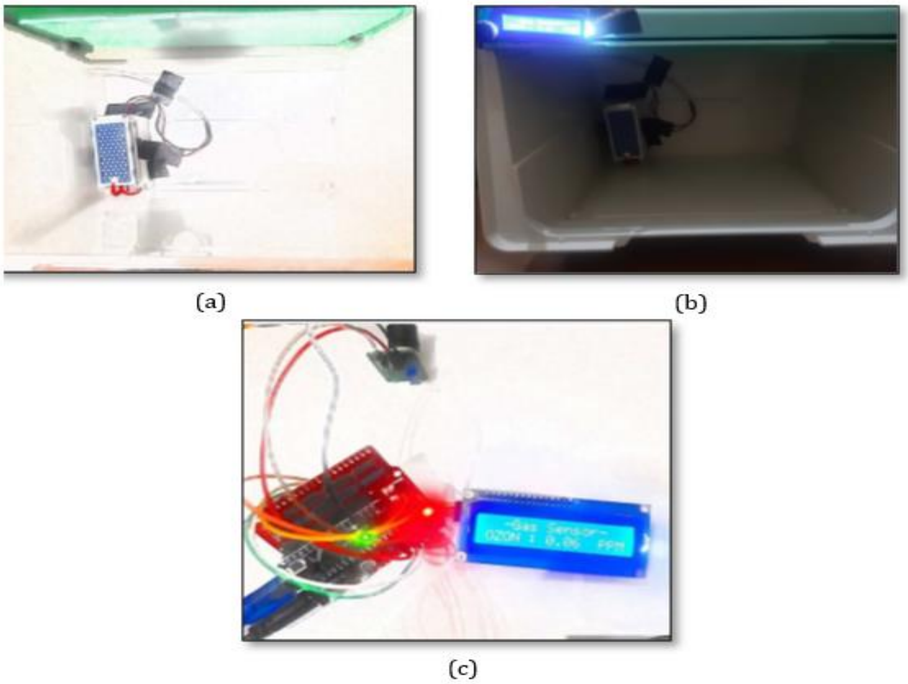


Figure 5 (a) Inside of the sterilization box with the generator. (b) Sensor MQ-131 outside the box. (c) Ozone sensor circuit MQ-131

3.3. Ozone concentration

Figure 6 shows ozone concentrations for (a) 5 minutes, (b) 10 minutes, and (c) 20 minutes in the sterilization ozone box. In Figure 6(a), the duration of the ozone generator is 5 minutes, the concentration of ozone formed reaches 1.65 ppm, and the duration of the decomposition of ozone into O_2 takes 3 minutes. For Figure 6(b) with the generator running for 10 minutes, every minute the ozone concentration increases linearly until it reaches its maximum point for 10 minutes with an ozone concentration of 4.01 ppm after the ozone generator is turned off and it takes 4 minutes to decompose all the ozone. Furthermore, in Figure 6(c), when the ozone generator is turned on for 20 minutes, the ozone concentration gradually increases until it reaches a concentration of up to 4.92 ppm. Then, to be able to decompose as a whole it takes about 6 minutes. Previous research conducted by (Ma'ruf et al., 2017) and (Waluyo et al., 2015) also showed this trend, in which ozone concentration will increase with the duration of treatment.

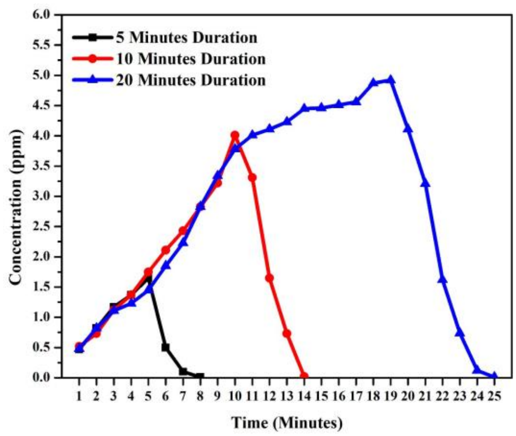


Figure 6 Ozone Concentration for: (a) 5 minutes; (b) 10 minutes; and (c) 20 minutes

In the three variations of duration, the increase in ozone concentration was stable until it reached a concentration of 4 ppm and slowed down. The concentration of ozone is known to be in line with duration and the increase of voltage to produce ozone (Vezzu et al., 2009). On the other hand, the concentration of ozone produced also refers to the oxygen around the generator. A previous study said ozone generators with a dry air source and an oxygen source directly experienced differences in the concentrations produced. Ozone produced by oxygen sources is more and more concentrated, as much as 7–10% (Teke et al., 2014). In this research, the source in the form of ambient air is not supplied with oxygen directly, and when it reaches a concentration of 4 ppm and a duration of 17 minutes, the increase in ozone concentration slows down. This is because the amount of oxygen that was in the sterilization box is decreasing, and because the box is closed, there is no incoming oxygen supply, so the oxygen concentration produced is stuck at four ppm.

3.4. Results of sterilization treatment of medical equipment

The results of the treatment process are presented in Table 1 that sterilizes several medical equipment's, namely a stethoscope and thermometer gun. The results showed that when each of these devices was not treated, bacterial colonies formed above 300 CFU, or it can be said to be Too Numerous to Count (TNTC). After that, each medical device was treated and put into a sterilization box for 20 minutes according to the optimal time in the previous experiment, then planted in two different media (PCA and MSA) and cultured; the number of bacterial colonies was reduced to below 50 CFU. It can be concluded that within 20 minutes, with the concentration of ozone produced, ozone can damage the walls of bacteria in each medical device and cause the bacteria to lyse or die. Previous research by (Botelho-Almeida et al., 2018), (Verma et al., 2016), and (Fu et al., 2020) with ozone treatment to reduce bacteria in medical devices was also successful.

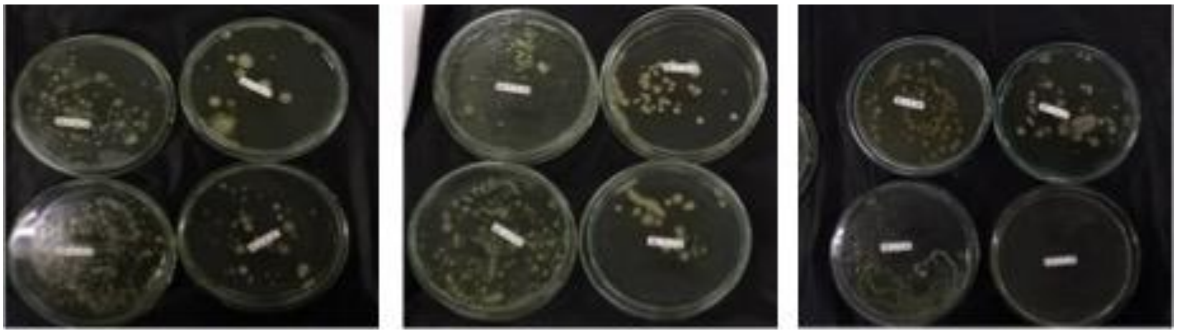
Table 1 Results of sterilization treatment of the medical equipment

Medical equipment	Duration (minutes)	Colonies formed (CFU) PCA	MSA
Stethoscope	0	TNTC	32
	5	126	26
	10	64	18
	20	36	10
Thermometer gun	0	TNTC	7
	5	134	6
	10	76	4
	20	36	1

Ozone is considered to be a disinfectant agent in the medical-related field for its bactericidal activity (Botelho-Almeida et al., 2018; Giuliani et al., 2018). The way it kills the bacteria is by disrupting the bacteria's cell membrane. Glycoprotein and glycolipid components of the cell membrane are the target of ozone properties, resulting in cell rupture. Protein and nucleotide disturbances are the other mechanism that occurs due to ozone exposure. Cellular enzymatic activity is affected by the ozone attack on the sulfhydryl groups of certain enzymes, leading to a loss of function. Ozone also attacks nucleic acid bases, resulting in DNA damage (Megahed et al., 2018). The previous study revealed the effect of ozone on membrane cell disruption leading to cell lysis in both Gram-positive and Gram-negative bacteria (Thanomsub et al., 2002).

Figures 7-8 show samples in PCA and MSA media after incubation. In PCA media, the growth of general bacterial colonies can be seen, while MSA media show certain bacterial colonies. MSA media is useful for growing bacterial colonies of *S. aureus* (Sharp & Searcy, 2006). There was a very significant decrease in colonies in the treatment with a duration of

20 minutes, Petri dishes given treatment in 5 minutes had the number of colonies TNTC (>300) on PCA media, while in MSA media colonies ranged under 50 colonies, this is because MSA is a selective and differential growth medium for Gram-positive bacteria, so that Gram-negative bacteria could not grow. When the duration of exposure is increased to 10 minutes, the number of colonies again decreases until only tens or dozens remain. At 20 minutes of exposure, it can be seen that the number of colonies decreases to near zero.



(a) (b) (c)

Figure 7 Bacterial colonies on PCA media after treatment for 5, 10, and 20 minutes (a,b,c)

(a) (b) (c)

Figure 8 Bacterial colonies on MSA media after treatment for 5, 10, and 20 minutes (a,b,c)

4. Conclusions

The sterilization box for medical equipment with an ozone gas leak sensor feature has been successfully developed. The sterilization box with an ozone generator has been designed with a MQ-131 ozone sensor as an indicator if there is a leak in the box. In testing the effectiveness of ozone treatment against *S. aureus*, the optimal duration was 20 minutes with an ozone concentration of 4.92 ppm, which significantly reducing the number of colonies compared to a shorter duration. Further testing was carried out on two medical equipment, namely a stethoscope and a thermometer gun, with a duration of 20 minutes, and the results showed very significant sterilization, with bacterial colonies formed after the treatment process being below 50 CFU.

Acknowledgments

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