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Efficacy of Dissolved Ozone against *Staphylococcus aureus* and *Bacillus cereus* Microorganism

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ABSTRACT

Ozone is a robust antimicrobial agent with numerous potential applications in the industry. Ozone, in either gaseous or aqueous phases is effective against the majority of microorganisms. Relatively low concentrations of ozone and short contact time are sufficient to inactivate bacteria and microorganism. This project investigated the efficacy of dissolved ozone against food-related microorganism. The ozone system was evaluated using the microbial effects on *Staphylococcus aureus*, and *Bacillus cereus* and its clinical efficacy against ORP level for disinfection was determined. The results showed that 100% of *S. aureus* and *B. cereus* were eliminated by the dissolved ozone in tap water. In conclusion, the dissolved ozone has great efficacy, lower cost and shorter disinfection cycle. Thus, this low temperature, ozone-based disinfection is a green technique and is regarded as one of the most promising disinfection methods.

Keywords: dissolved ozone; bacteria; disinfection

INTRODUCTION

Foodborne diseases that result from the food spoilage or contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, are a major concern worldwide. In this context, food-related microorganisms, *Staphylococcus aureus (S.aureus),* and *Baccilus cereus (B. Cereus)* are the main microorganisms related to the food safety in terms of frequency and seriousness of the disease (Umar et al., 2013). The bacteria cause two types of gastrointestinal disease, the diarrheal and the emetic syndromes, which are caused by very different types of toxins (Ehling-Schulz et al., 2004). Moreover, the spread of the bacteria is caused by improper disinfection of hand wash.

Over the past few decades, numerous studies for inactivation of various pathogens and microorganisms by various disinfecting agents, including ozone, chlorine dioxide, photocatalytic reaction, free and combined chlorine and short-wavelength UV light have been conducted (Cho et al., 2010; Li, et al., 2011; Van Haute, et al., 2013; Huda, et al., 2019). The primary focus of most studies was providing the information on the disinfectant doses and contact times required to effectively control pathogens. However, the mechanisms of microbial inactivation are relatively poor established (Dodd, 2012).

Among those methods, ozone showed promising results with chemical-free and easy procedure. Ozone is known as a powerful oxidant the disinfection potential of which is widely used in the food industry, medicine and dentistry and wastewater treatment (Kim et al., 1999; Seidler et al., 2008; Tango and Gagnon, 2003; Khadre, et al., 2001; Kumar, et al., 2009; Jurczyk, et al., 2019). Ozone is potentially useful in decreasing the microbial load, the level of toxic organic compounds, the chemical oxygen demand, and the biological oxygen demand in the environment (Azarpazhooh and Limeback, 2008).

Ozone, is commonly dissolved into water for water treatment, or for water to carry ozone for disinfection and sterilization. In fact, many of the ozone applications require it to be dissolved into water. As ozone is partially soluble into water, it is possible to dissolve ozone , but the process require the proper equipment to be more efficient. The solubility of ozone into water is based on water temperature, ozone concentration, and water pressure. The process of dissolving ozone in water is optimized by maximizing the surface area between the gas and the water. This is commonly done by using bubble diffusers, venturi injectors and turbine mixers (Hassan and Hawkyard, 2007).

However, ozone is relatively unstable in aqueous solutions. It decomposes continuously, but slowly. The half-life of ozone in distilled water at 20°C is generally considered to be 20 to 30 min (Mendes et al., 2007). Therefore, ozone must be generated and used straight away in the disinfection process.

In order to measure the efficiency of the dissolved ozone, Oxidation reduction potential (ORP), electrochemical cell and "Indigo" test can be used. The ORP reacts to any oxidizing influences in the water. ORP is simple, rugged and inexpensive, offering many advantages to real-time monitoring and recording of water disinfection potential, a critical water quality parameter. An ORP probe attached to a meter is placed in the water sample, giving the ORP readout in mV (Silindir and Ozer, 2009). This method has also been used to check the antibacterial potential of the ozonated water (Fitria et al., 2019).

In this paper, the system investigates the efficacy of dissolved ozone in tap water against *S. aureus* and *B. cereus* microorganisms. The ozone utilizes low temperature with high sensitivity. Ozone disinfection is carried out through oxidation, a process that destroys the organic and inorganic matter. In this process, a generator is used to convert oxygen to ozone, as a 6 to 12 percent concentration of ozone continuously flows through the chamber. The ozone will dissolve into tap water and the ORP level of 500 to 700 mV are recorded to observe the dissolved ozone reaction to the microorganism.

MATERIAL AND METHODOLOGY

Ozone – based system

The experimental setup of the system is as shown in Figure 1. The ozone is produced using a generator to be supplied to the tap water in the tank. Then, the ozone and water were mixed in the mixer tank through a venturi injector and static mixer until it reached the required ORP value as well as pH value to be analysed. The utilization and handling of ozone should be done safely. Any excess ozone was reverted back to normal oxygen using an ozone destructor making it virtually, non pollutant.

The system is fully automated controlled using the Arduino software (IDE). The Arduino is an easy-to-use hardware and software. Arduino also provides a simple and a clear programming environment for implementations. The C language used in the development were compiled using the Arduino software (IDE). The entire component such as relay, buzzer, LED, water sensor and ORP meter adapter were connected to the controller box, as shown in Figure 1.

The operation of the ozone-based system is shown in Figure 2. The process control of ozone-based system started when the system is ON, the tank is filled up with 25 liters of tap water. Meanwhile, the water sensor will read the level of water in the tap water tank. If the water level is low, then the buzzer is turned 'ON' to start the ozonation process. Afterwards, the water was pumped through an ozone injector. In this project, the venturi injector was used as a method of an ozone injection. The venturi injector is a crucial part to maximize the percentage of ozone transfer into the tap water.

Next, the ozonated water flowed through a static mixer before it was automatically pumped into the contact mixer tank. During this process, the ozone gas was mixed with tap water to dissolve into tap water, thus allowing the water pressure to be maintained. The ORP meter probe from Horiba U-50 series is used to show the ORP reading in mV. The sample of ozonated water that had been set having the value of ORP reading of 500 mV, 550 mV, 600 mV, 650 mV and 700 mV were taken. Each water sample with different

Figure 1. Experimental setup

ORP level was then mixed with cultured bacterial liquid sample to observe their efficacy in killing the bacteria. The process was repeated for another sample of cultured bacterial liquid.

Bacterial Test

The bacterial strains were diluted with a serial dilution method to obtain a $1x\,10^{-6}$ dilution. In order to study the efficacy of dissolved ozone in killing the selected bacteria, 0.1 ml of bacterial liquid of culture was mixed with 0.9 ml of ozonated water into an Eppendorf tube follow the level of ORP reading which has been set from 500 mV to 700 mV. The controlled tap water suspension was constituted by 0.1 ml for each type of bacteria and similar to mixed with 0.9 ml tap water. The sample was spread onto the agar plate by using a pipette tip. Afterwards, the sample was incubated in an incubator chamber at 30^{o}C. Next, after growing for 24 h, the number of colonies in each agar plate was calculated using Eq. (1).

$$\text{Killing rate (\%)} = \frac{\text{control colony-tested colony number}}{\text{control colony number}} \quad (1)$$

Eq. (1) Killing rate for microorganism

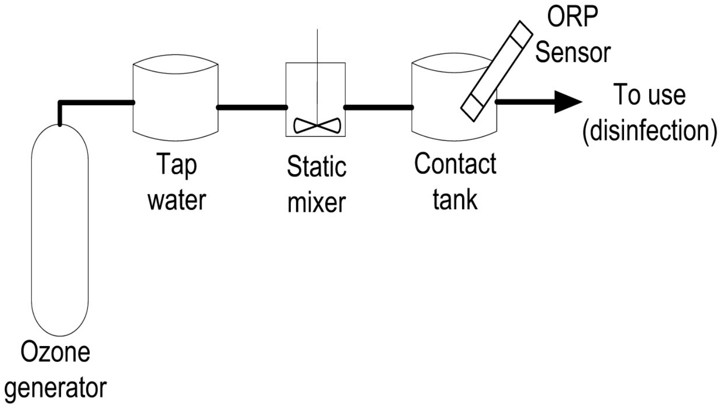


Figure 2. Process of the ozone-based system

RESULTS AND DISCUSSION

The efficacy of ozonated tap water with different level of ORP reading (500, 550, 600, 650, and 700 mV) in killing the bacteria in tap water was analysed through exposure of the microorganism of *S.aureus,* and *B. Cereus* to the dissolved ozone in tap water (ozonated tap water). An analysis was conducted to observe what ORP level is optimum for the dissolved ozone to inhibit the microorganisms effectively.

Table 1. Bacterial test on the controlled and ozonated tap water

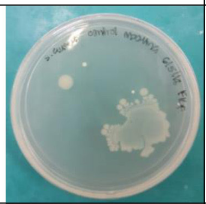
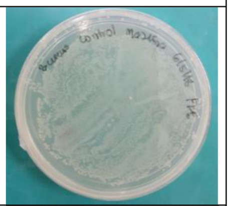
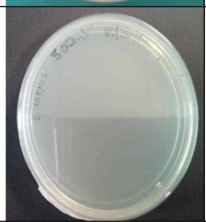
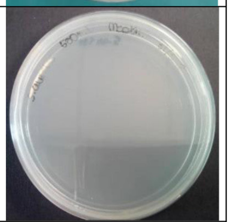
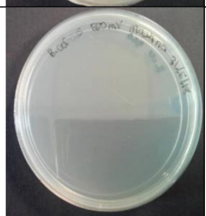
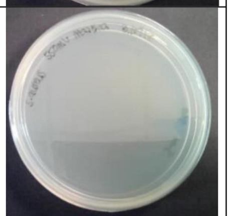
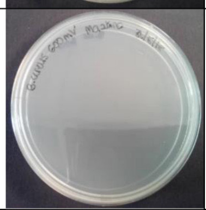
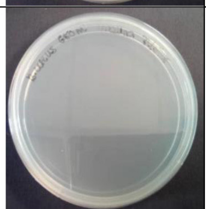
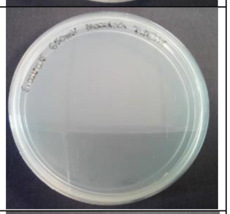
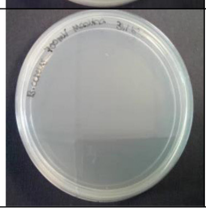
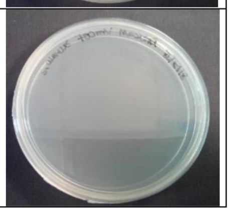
ORP level (mV)	Bacterial Test	
	S. aureus	*B. cereus*
Controlled		
500		
550		
600		
650		
700		

Table 1 show the result of the samples after incubation.

This test study was carried out to determine whether the ozonated tap water with different level of ORP reading has effective disinfecting action on the deposited bacteria which are *B. cereus* and *S. aureus* after being sprayed on the agar surfaces in Petri dishes. As can be seen from Table 1, the *B. cereus* and *S. aureus* bacteria can be clearly observed on the surface of the agar sample of untreated tap water. It means that the *B. cereus* bacteria and *S. aureus* bacteria can grow well in this type of environment.

The first sample of dissolved ozone with ORP reading of 500 mV mixed with agar surfaces in petri dishes showed the absence of both *B. cereus* and *S. aureus*. Similarly, the sample applied with 550, 600, 650 and 700 mV showed clear surfaces in petri dishes indicating that the bacteria were completely killed. There are clearly no signs of colony-form or any bacteria growing in petri dishes mixed with the ozonated tap water samples for both types of bacteria in every ORP level set as above.

The bacteria were counted and compared with the ORP level and controlled condition respectively, using colony-forming unit to calculate the killing rate. The colonial test of the controlled sample showed the existence of colony-forming in both samples of *B. cereus* and *S. aureus* bacteria. Conversely, the sample applied with ozonated tap water showed no colony-forming of both bacteria sample at any level of ORP. The result of the killing rate of the samples is as shown in Figure 3.

In this study, it was shown that the ozonated water with ORP reading above 500 mV has strong in vitro antibacterial effect against *B. cereus*, and *S. aureus*. From the data shown, the ozonated water (1 ml) was capable to sterilize 100 % of *B. cereus*, and 100 % of *S. aureus* in 24 hours. This result indicates that ozone disinfection has very powerful antimicrobial effect against gram negative and positive microorganisms, which was confirmed by the digital microscope view of ozonated water.

The results indicate that the ORP value of 500 to 700 mV shows an excellent effect in killing all the bacteria. However, in order to achieved promising and excellent result in all applications, 700 mV is set as the optimum ORP level of disinfection. It is believed that at this level, all the bacteria are confirmed killed.

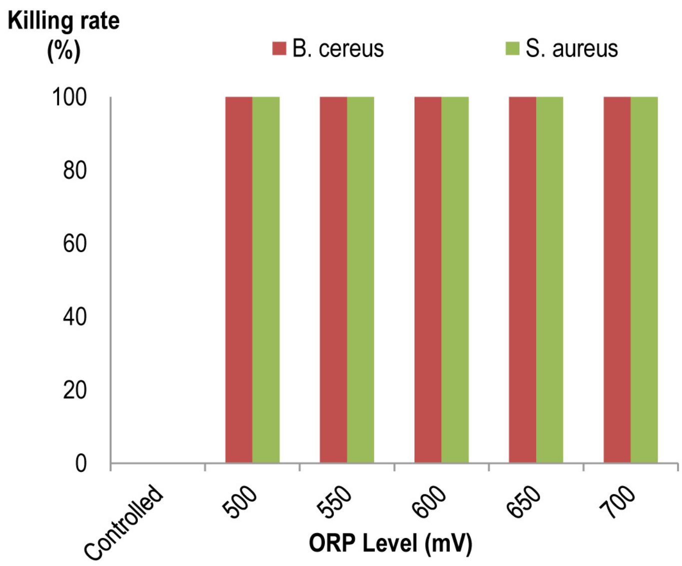


Figure. 3. Killing rate

CONCLUSION

In conclusion, the dissolved ozone in tap water shows an effective way in removing and simultaneously destroying the microorganisms. The results showed that it can effectively kill 100% of *B. cereus*, and *S. aureus* at ORP level as low as 500 mV of dissolved ozone. However, for future industrial applications, the ORP level of 700 mV is recommended as the optimum level for the disinfection. It was proven that dissolved ozone in tap water can be a powerful medium and high efficacy in disinfecting bacteria, which has 6 times the oxidizing power of chlorine and other substances.

Acknowledgment

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Research Article

Antibacterial Potency of Ozonated Water against *Escherichia coli*

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Abstract

Sterilization is essential for inactivation of microorganisms. There are many methods of sterilization, such as the use of heat or chemical processes. However, some equipment can be damaged by heat and can only be sterilized at low temperatures. Failure to properly disinfect or sterilize equipment may lead to transmission via contaminated objects. This paper presents a sterilization process using ozonized water at a temperature of 29.5°C with gram-negative bacteria (*Escherichia coli)*. The antibacterial effect was examined with various concentrations of ORP (oxidation reduction potential) at 702 mV, 802 mV, 940 mV, 950 mV, and 960 mV. A strong linear correlation was observed between ORP value and the surface area of the antibacterial effect. It was found that increasing the concentration of ORP affects the surface area of *Escherichia coli*.

Keywords: Antibacterial, *E. coli*, ozonated water, oxidation reduction potential.

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INTRODUCTION

Sterilization is important for inactivation of microorganisms, providing improved good quality of life for humans. Inactivation of a microorganism includes its destruction or elimination by physical or chemical processes or a combination of the both[1]. Application of conventional sterilization methods, such as using high temperature, high pressure, chemical gas, and radiation (gamma rays), depends on the type of materials being sterilized.

Although some methods using high temperature and high pressure are effective in inactivating microorganisms, they are inefficient regarding sterilization duration, energy consumption, and plastic equipment application[1,2,3]. Sterilization using chemicals, gas, and radiation can be applied to many types of equipment. However, the method will produce toxic residues, change molecular structure (cross-link or scissor), release odors, change pH, cause discoloration and degradation of a few materials, or affect bond strengths and change over the shelf life of the material[3].

Another way to sterilize is through the use of ozone as a disinfectant. Use of ozone and ionized water is an environmentally friendly method for inactivation of bacterial[4,5] . Ozone is a powerful and effective germicidal oxidant that has great potential over chlorine (chemical method) and other disinfectant methods. Ozone is currently used as a disinfectant for water, air, and various pharmaceutical applications[6,7,8]. The most common method for ozone generation is Dielectric Barrier Discharge (DBD). The DBD model is shown in Fig. 1. This method consists of at least one insulating layer between two electrodes or cylindrical electrodes that connect to an AC power supply with a dielectric layer.

Homogeneous discharges produced in the air gap between the electrodes–the volume of the reaction chamber–cause the temperature in the chamber to remain low (25°C), which reduces the need for a cooling system[9,10,11]. Dry air or oxygen that is supplied in the DBD chamber allows the dissociation of oxygen molecules to form ozone. In 2001, ozone in the gaseous and aqueous phases was accepted by the U.S. Food and Drug Administration (US FDA) as an antimicrobial agent for the treatment, storage, and processing of foods[12].

Ozone has decomposition products which can rapidly inactivate microorganisms (e.g., hydroxyl radical) by reacting with intracellular enzymes, nucleic material, and components of the cell envelope[12]. Inactivation of bacteria using ozone causes leakage of inner contents due to oxidation of unsaturated lipids in the cell envelope, which finally results in cell lysis[12,13,14]. The mechanism most referred to in the formation of ozone in electrical discharge is the following
chemical reaction[15,16,17,18].

$$e^- + O_2 \rightarrow 2O + e^- \quad (1.1)$$

$$O + O_2 + M \rightarrow O_3 + M \quad (1.2)$$

M is a third component necessary to support the reaction when the air is injected. M could be gas molecules such as oxygen, helium, or argon.

In the reaction, electron bombardment breaks oxygen molecules apart; electrons that avalanche will recombine with each other or with the other oxygen molecules for ozone formation[15]. The method used in this experiment aimed to detect the extent that dissolved ozone mixed with distilled water was monitored through the ORP meter. ORP is well-known to have higher efficiency in inactivating bacteria with higher values[19].

Failure to properly disinfect or sterilize equipment may lead to transmission via contaminated objects. The objective of this study was to observe whether the ozonized water with different concentrations of ORP could develop an inhibition zone for *Escherichia coli.* The linear

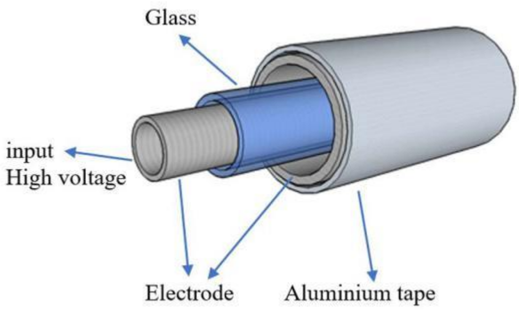

Fig. 1. Dielectric Barrier Discharge model. [Change aluminum to aluminum. Change to High voltage input]

correlation between surface area and rate of oxidation- reduction potential used in ozonized water was examined.

MATERIALS AND METHODS

Escherichia coli ATC9222 was used as a sample for indicating contaminated objects. At first, the *Escherichia coli* was developed in Nutrient Agar slant and then transferred to a nutrient broth composed of 0.65 g and 50 ml of distilled water, and incubated for 24 hours at 35°C. Samples in the nutrient broth that were grown for 24 hours were spread into a petri dish (Anumbra, 100 x 15 mm_2). Whatman papers were placed into the petri dish that already dyes into the ozonized water. The results were obtained after the petri dish incubated for 24 hours at 35°C.

Ozone was generated using a dielectric barrier discharge system with an applied voltage of 15 kV at atmospheric pressure. Oxygen with a purity of 99.9% was injected into the ozone generator at a constant flow rate of 0.5 l/min. Electron avalanches processed in the electric field led to the creation of partially ionized plasma and created ozone. Ozone was mixed with distilled water by using a Venturi Injector and a static mixer. From the static mixer, the ozonated water was collected in a bottleneck tube. The ozonated water was measured by an ORP meter (AZ Instrument 8551) and a pH meter (Hanna HI 98107) to analyze the correlation of ORP and pH with the surface area containing *Escherichia coli* in the petri dish (Fig. 2).

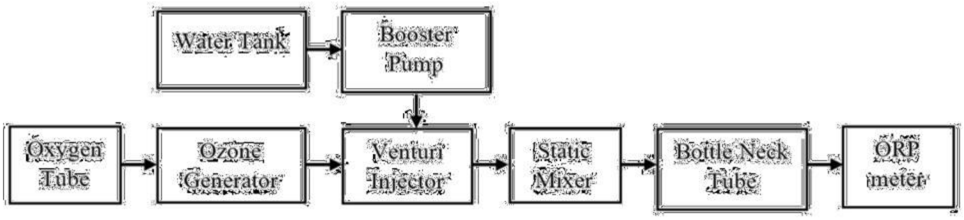

Fig. 2. Experimental setup

The ozonated water in various concentrations of oxidation-reduction potential (702 mV, 802 mV, 940 mV, 950 mV, and 960 mV) was injected into Whatman papers and then put into a petri dish already containing *Escherichia coli* in EMB (eosin methylene blue) media. Formed inhibition zones were obtained after the petri dish was incubated for 24 hours at 35°C. The circular transparent zones in the exposed samples represent the growth of the inhibition zone. They could be observed directly without special equipment and the diameter measured by using a ruler (1 mm precision).

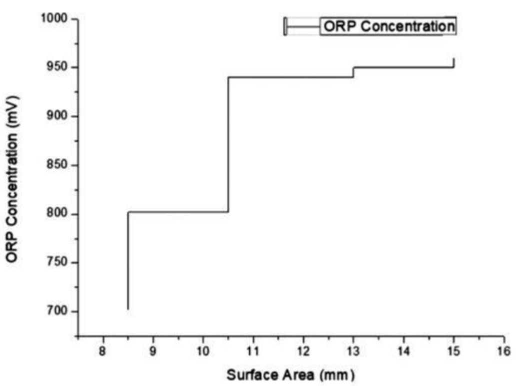

Fig. 3. ORP concentration vs surface area

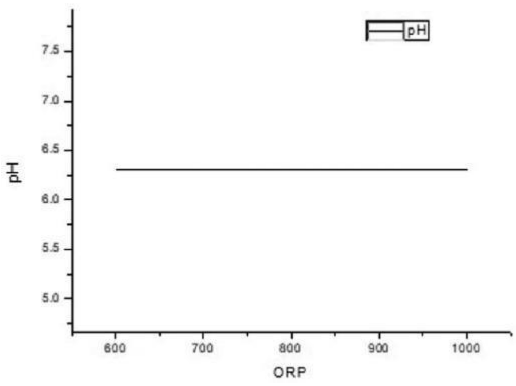

Fig. 4. pH vs ORP concentration

RESULTS AND DISCUSSION

The addition of ozone to the distilled water gives a relatively linear result of changes in the surface area with the different oxidation-reduction potential concentrations used. Fig. 3 provides information on the antibacterial effects on *Escherichia coli* tested with ORP.

The width of the surface area in the *Escherichia coli* stained with EMB shows a beneficial contribution of ozonated water to bacterial growth elimination. The observation of an inhibition zone against bacterial growth was carried out in various concentration over 3 days. At a low concentration of 702 mV, as shown in Fig. 3, the inhibition zone was limited only to the surface area, even in 3 days of observation. As shown in Fig. 4, when using a concentration of 960 mV, the inhibition zone was more significant than in lower concentrations, and no modification was seen in 3 days. This proves that high concentrations of ozonated water can create a beneficial inhibition zone against *Escherichia coli*, as shown in Fig. 5.

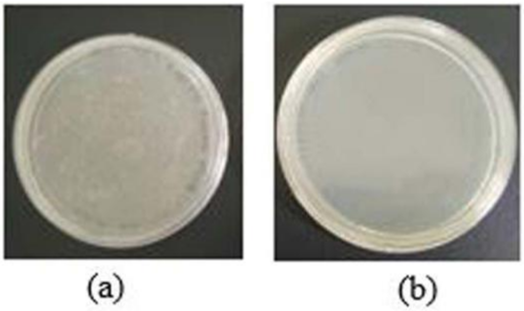

Fig. 5. a. Before treatment**;** b. After treatment

The ORP value gives the potential redox level of the ozonated water, which has an oxidation value that acts to inhibit and inactivate bacteria. During the inhibition process, the ORP value indicates the oxidative agents whereas pH remained stable. This result agrees with the findings of Wang *et al*.[20], oxidation was not influenced by low pH, suggesting that oxidation plays an important role during the process. Based on Fig. 6a and Fig. 6b, ozonated water is able to inactivate *Escherichia coli*, there was no growth after incubation for 24 hours at 35°C. This result has a good agreement with evidence that ozone is known to have antibacterial activity[21,22] (Fig. 5).

Additional distilled water creates an electron impact reaction in the impulse stage, also generating H and OH radicals that are able to inactivate bacteria[16]. The ORP was considered to be a more suitable indicator for the optimal operation of the antibacterial process (Fig. 6a and Fig.6b).

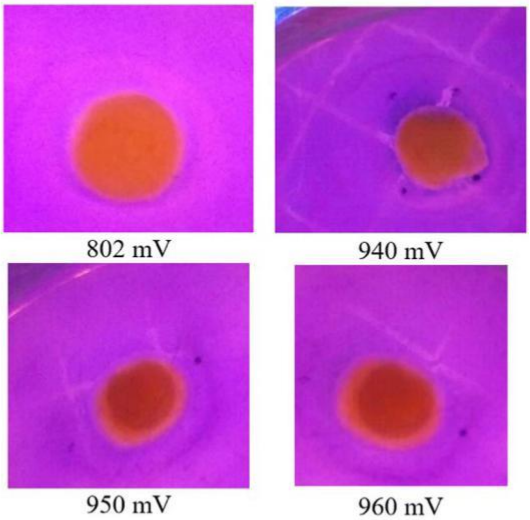

Fig. 6. Inhibition zones against *Escherichia coli*

CONCLUSION

Ozone is known as an antibacterial agent. Ozone mixed with distilled water, that is, ozonated water, has good results for increasing the inhibition zone against *Escherichia coli*. The ORP concentration in ozonated water plays an important role as an oxidative agent for inhibition and inactivation of bacteria but the pH value was almost stable in the entire process. Since, the electron that oxidized in water could affect bacteria growth around surface area.

Further research is needed on the oxidation-reduction potential for other bacteria to determine the inhibition zone at various concentrations and subsequent cell damage after exposure to ozonated water. The proposed system may find future use as a means for sterilizing medical equipment using ozone generated and mixed with distilled water.

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COFLICT OF INTEREST

The author declares that there is no conflict of interest.

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Comparison Double Dielectric Barrier Using Perforated Aluminium for Ozone Generation

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***Abstract*- Ozone generation has widely known–may replace chlorine compounds in various applications including wastewater treatment, polluted air processing, antimicrobial, bacterial inactivation, semiconductor oxidation, and serve as disinfectan. This study mainly focuses on comparison of different dielectric materials performances using perforated aluminium to obtain high concentrated ozone. Perforated aluminium with sharp edges used for ozone generation as electrode. Dielectric barrier discharge (DBD) using glass and 96% alumina ceramic have been chosen for limiting discharge current due to its low thermal conductivity and low dielectrics loss when high breakdown voltage occur. Double dielectric barrier using perforated aluminium has been observed using 96% alumina and quartz glass, both within 2 mm thickness. Ozone concentration of alumina ceramic dielectric for 0.5mm space gap was higher than quart glass. However, for 1mm space gap, ozone concentration using quart glass was higher than alumina ceramic. These results lead to optimum condition for DBD using alumina ceramic is not more than 0,5mm space gap.**

Index Terms—Dielectric Barrier Discharge, Ozone, Dielectric Material.

I. INTRODUCTION

Ozone is a powerful oxidizing agent made of stable molecular oxygen (O2) that can replace chlorine compounds in various applications including wastewater treatment, polluted air processing, antimicrobial, bacterial inactivation, semiconductor oxidation, and as a disinfectant [1][2][3][4]. However, the remaining of ozone will return to natural oxygen that makes environment unaffected by pollution from its byproducts [5].

Ozone is unstable therefore it may decompose gradually (in minutes) at room temperature and quickly (<1 second) at higher temperatures [1]. Due to this instability, ozone has to be made in place for various desired applications. Based on ozone capabilities that recognized, this research on ozone continues to be improved.

Ozone may be produced by several methods such as electrical discharge, optochemical reaction and UV radiation. Electrical discharge is the most generally used method of ozone generation. Among them are corona discharge, gliding arc discharge and dielectric barrier discharge. The most commonly used method for ozone generation is the dielectric barrier discharge (DBD) that operated at atmospheric pressure [6][7][8]. This structure consists of two separated electrodes with gap distances on either or both sides using a dielectric material. This system produces stable non-thermal plasma and under atmospheric pressure so it is a good method for ozone production [9] [10]. This method was also well-known that the most cost effective method[11].

The dielectric barrier that contained above the grounded electrode is used to limit the discharge current, prevents the transition of the DBD discharge to an arc discharge, to make sure that stable non-equilibrium plasma can be generated even under atmospheric pressure, that is appropriate for ozone generation [12][13][14][15]. Because of that, dielectric barrier that contained above grounded electrode may serve to distribute the discharge evenly throughout the electrode path area and increase ozone generation by using low-conductivity dielectric material. In addition, ozone generation may also be affected from gap distances, electrode material, gas type, voltage, power supply, gas input and dielectric material [16][17][18].

The method of ozone forming may dissociate the oxygen molecules into atoms so that oxygen molecules and third particle (usually oxygen or nitrogen if air is injected) collide and form ozone immediately. The ozone formed would have high-energy electrons between 1-10 eV that generated in discharge removal area of DBD [10]. Ozone formation mechanism of ionization and recombination includes both dissociation and association [19]. The processes start by electron bombardment of O_2 molecules in the discharge region

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resulting in oxygen atoms and combine the electron with oxygen molecules to create ozone. The major reactions for ozone formation are showed as follows:

$$e + O_2 \rightarrow O + O + e \quad (1)$$
$$O + O_2 + M \rightarrow O_3 + M \quad (2)$$

Where M is a third collision partner that has a role in the process of energy absorption but does not react to chemistry so that the distribution of high energies electrons determines the amount of ozone formed. At the same time the formation of ozone occurs with the occurrence of ozone decomposition. The decomposition of ozone takes place through the reaction as follows:

$$O + O_3 \rightarrow O_2 + O_2 \quad (3)$$
$$e + O_3 \rightarrow O_2 + O \quad (4)$$

From Eq. (1) to Eq. (4) show that opposition between ozone generation and ozone decomposition around process ozone production [11].

Material dielectric using glass and alumina that have been known for low conductivity and low temperature plasma that enhance ozone generation efficiency [7]. The objective of this study is to investigate the optimum conditions for the effective ozone concentration in dielectric barrier discharge, measurements of ozone concentration using perforated aluminium were carried out for various conditions of gap spacing, dielectric material and voltage.

II. Experimental Setup

The experimental setup used to study the ability of a perforated aluminium electrode to generate ozone. The plane-plane spesification of perforated aluminium electrode sheet are 1.5 mm thickness, 1 mm diameter hole. Gap distances between dielectric barriers are 1 mm and 0.5 mm. This study using variation of dielectric barrier which are glass and alumina ceramic for double dielectric barrier which have known for their lower dielectric breakdown [20]. Dielectric barrier procedure was assumed, with both sides having one perforated aluminium electrode sheet (7.7 cm length and 7.7 cm width) located between aluminium foil and dielectric barrier (Fig.1). The aluminium foil serves as high voltage electrode on one side of reactor chamber and as ground electrode on the other side.

Glass sheet within 2 mm diameter was used as the dielectric barrier. The working gas (99.99% pure oxygen) with flowrate 1 L/min was injected through plasma chamber with ambient temperature and atmospheric pressure. Input voltage 18 kV and 19 kV at frequency of 50 Hz used to supply the reactor chamber. Dielectric barrier discharge (DBD) which using 50 Hz power source is a suitable choice [21].

The advantages of using low frequency include limiting current of the dielectric layer to the electrode effectively thereby stabilizing the discharge, and the availability of inexpensive and highly efficient solid-state power supply power supply [13].

High voltage probe *(Tektronix P6015A, 1000:1,3.0 pF, 100 MΩ)* for measuring input voltage was connected in parallel with the DBD reactor. The voltage applied to the electrodes was measured via voltage divider. The transported charges were measured by placing capacitor 0.22 mF between the grounded electrodes and ground, respectively. The voltage waveform were recorded by Picoscope 3206B (200 MHz, 500 MS/s).

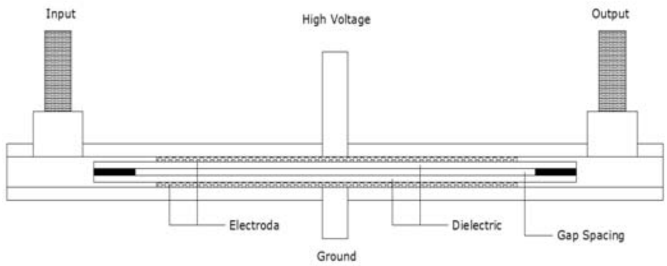

Figure 1. Dielectric Barrier Discharge Chamber (DBD)

Two parallel-perforated aluminium electrodes were placed on each side of the dielectric with barrier alumina 96% or glass. The alumina had an Al2O3 content of 96%, thermal conductivity 27,3w/m.k; insulation resistance of 22.5 kV, alumina ceramic has characteristic of high performance to achieve thermal efficiency. An electrode was connected to high voltage side and the other was connected to ground via a capacitor of suitable capacitance.

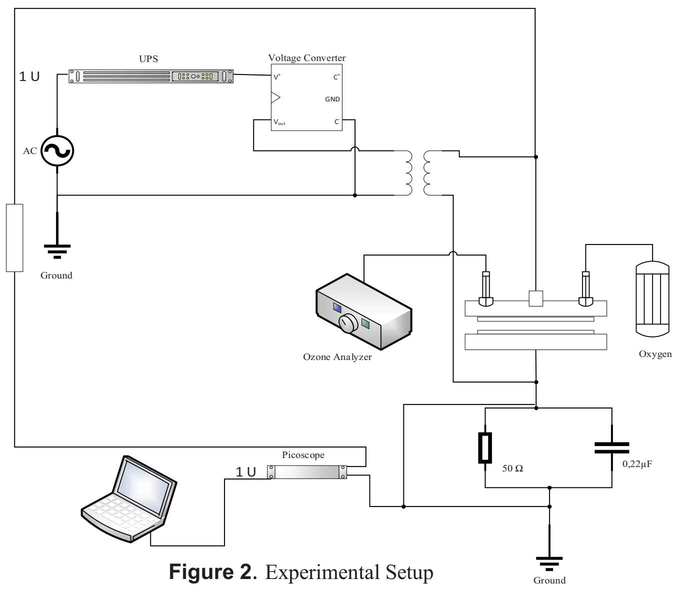

Figure 2. Experimental Setup

A 50-ohm current-limiting resistor was connected between output of transformer and reactor. The gas flowrate was maintained at 0.1 L/m and the pressure in the reactor was controlled by the needle valve in ozone analyzer (BMT 964) and also measured the outlet ozone concentration. Ozone generation was conducted using a planar type chamber made by acrylic or polymethyl methacrylate and using perforated aluminium as electrode with glass and alumina ceramic as the dielectric material. This system was operated at atmospheric pressure and ambient temperature. The advantages of planar chamber shape are easy modification for air gap with filler materials, simple construction, simple replacement and arrangement for different type electrode and dielectric [20].

III. RESULTS AND DISCUSSION

Dielectric material used may affect high ozone produced and ozone concentrations as it allows to contribute to the limitation of rising gas temperatures in the discharge area requiring dielectric materials that have low thermal conductivity and dielectric constants. Based on Sung et.al, using 1 mm thick quartz glass disk (Q) as dielectric material with relative dielectric constant of about 3.8 and 1 mm thick alumina disk (ALO) with relative constant dielectric about 8.5 using the oxygen and dry air having constant flow rate of 3L / min. The ozone concentration obtained with the alumina disk is higher than the quartz glass one, as each length of the slit decreases by increasing discharge voltage. Thermal conductivity of alumina disk is about 15 times bigger than quartz glass. Previous case with alumina disc, relatively high ozone product obtained only when the length of short gap [16]. However, it needs more investigation about dielectric material that may be used for ozone generation and ozone yields.

This experiment using perforated aluminium that would be better in ozone generation due to its sharp-edged holes, which makes electric field strength in discharge region become high. Based on Buntat et.al, to produces improve glow discharge stability at atmospheric pressure by using the discharge configuration with perforated aluminium electrodes that means effective of improving the ozone generation [22]. However, perforated aluminium using different double dielectric (glass and alumina ceramic 96%) has been observed, respectively.

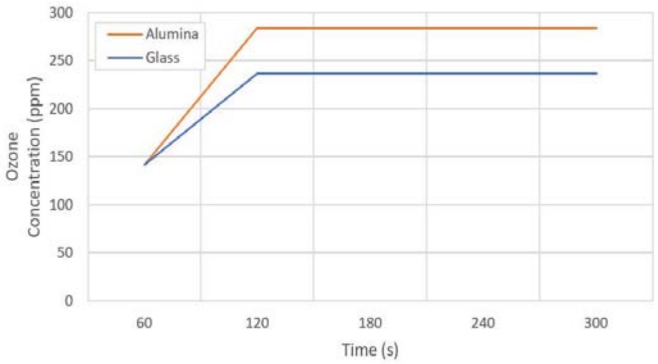

Figure 3. Ozone concentration using alumina and glass in 0,5 mm space gap at 19kV input voltage

Ozone concentration obtained from dielectric materials (glass and alumina Ceramic 96%) shows inversely proportional result when using different gap length between dielectric. For glass dielectric, optimum ozone concentration obtained 236.5 ppm with 0.5 mm and 1 mm gap length. It different with alumina dielectric that ozone concentration obtained 283.8 ppm with gap length 0.5 mm (Fig. 3) and 141.9 ppm with gap length 1 mm (Fig.4).

These result inversely proportional when gap spacing changed from 0,5 mm to 1 mm. At the same condition using perforated aluminium and glass dielectric has higher ozone concentration than alumina dielectric within 1 mm space gap. However, when alumina dielectric was used, ozone concentration become higher than glass with 0,5 mm space gap.

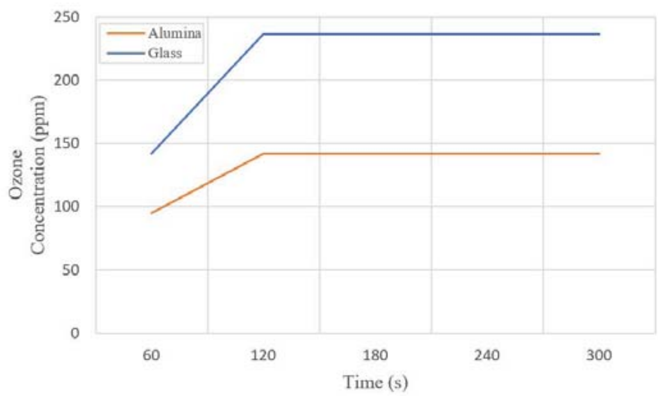

Figure 4. Ozone Concentration using alumina and glass in 1 mm space gap at 19kV

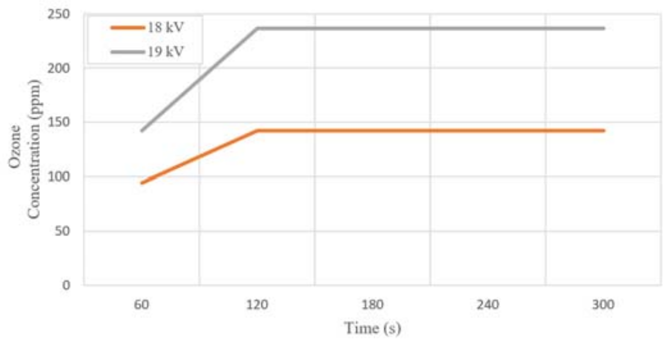

Figure 5. Ozone Concentration using alumina in 1 mm gap spacing at 18 kV and 19 kV

These indicate alumina has good condition for ozone concentration when using gap spacing lower than 1mm. These results in accordance with Sung et.al who used alumina disk, then ozone concentration decreased as gap length getting longer. Dielectric material has consumed power that converted into heat whereby not only the temperature in the dielectric material but also the temperature in the discharge region increases. Sung et.al assumed that discharge power became high in conditions where alumina disk of which the dielectric constant was larger than that of the quartz disk was used as a dielectric material even under the same discharge condition. The discharge power increased as the increasing of discharge voltage and the gap length [16]. Increasing the gap space may increase the power consumption of the system [23]. These mean that ozone concentration increases when decreasing discharge power and gap length.

One of factor that may affect high ozone concentration is limitation of ozone dissociation at discharge region; another factor is the gap distance between dielectric materials. The dissociation reaction of oxygen and ozone molecules, by the impact of electrons, depends on the reduced electric field (E/n, where E is electric field and n is particle density) [16]. Ozone dissociation process may affect decomposition ozone rapidly.

Based on these results, applied high-voltage will increase ozone concentration (Fig.5). The increasing voltage will increase ozone concentration due to high voltage applied to dielectric barrier discharge. Then these lead to high electron avalanche recombined with other molecules that formed ozone and high electronegativity of oxygen in which play important role in this observation.

The result shows that ozone concentration obtained 236,5 ppm and 283,5 ppm is suitable for home usage, sterilization vegetable with ozone concentration in this range. Zhao et al used gaseous ozone (6.7 ppm for 6 h) as a disinfectant in reducing microbial populations in ground black pepper, observing a 3–6 log reduction depending on the moisture content material [24]. Selma et al suggested that the ozone treatments at levels of 1.6 and 2.2 ppm for 1 min reduced Shigella sonnei population in water by 3.7 and 5.6 log CFU ml^{-1}[25]. Najafi et al assumed for reducing both coliform and Staphlyococcus aureus populations on date fruits which minimum of 1h ozone treatment at 5 ppm could be effectively used, however for elimination of the total mesophilic bacteria as well as yeast/mould present that still need longer exposure times were required [26]. Cullen et al have study that using ozone 5 ppm ($p < 0.05$) in 60 min for Escherichia coli and S. aureus were not found on cultured plates inoculated with the treated samples after treatment. There are most studies have been conducted for Gram-negative bacteria (E. coli), which give the impression to be more resistant than Gram-positive bacteria. Bacteria are also more sensitive than yeasts and fungi [27]. However, based on those all studies that ozone is safe for food due to one of potential advantages is that excess ozone auto-decomposes rapidly to produce oxygen and thus leaves no residues in food.

IV. CONCLUSION

Dielectric barrier discharge using alumina and glass has been conducted in this paper. The optimum conditions for the effective ozone concentration in dielectric barrier discharge, measurements of ozone concentration using perforated aluminium were carried out for various conditions of gap spacing, dielectric material and voltage. The use of perforated aluminium as sharp edges electrodes was to increase ozone concentration. It was found that maximum ozone concentration 236,5 ppm when using glass with 1 mm gap spacing and 283,5 ppm when using 236,5 ppm using alumina with gap spacing 0,5 mm. This result suitable for home usage and sterilization vegetable with ozone concentration in this range due to one of the potential advantages is that excess ozone auto-decomposes rapidly to produce oxygen and thus leaves no residues in food.

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