Efficiency Evaluation of Developed Ozone System in Microorganism Treatment

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Ozone generator system using discharge coronas was fabricated to produce ozone gas and ozonated water of varying concentration. The generated ozone air emitted to the water through bubble diffuser or venturi injection depending on the input air mass transfer rate. The concentration of ozonated water was examined using both ozone test strip and Schoenbein paper. It is found that the production of ozone water by using bubble diffuser technique facilitates the production of higher concentration of ozonated water with higher ozone transfer efficiency. The efficiency of ozone gas in microorganism treatment is verified through the lowest number of microorganism's colony available compared with other treatment methods. The half-life of ozonated water in this study was improved by increasing the transfer rate of water and solubility of ozone in water. The concentration of ozonated water was found increased through the application of pressurised oxygen input gas.

Keywords: ozone, ozonated water, microbial reduction, ozone half life

I. INTRODUCTION

Ozone is a naturally occurring gas in the atmosphere. Each ozone molecule contains three atoms of oxygen. In general, there are two basic methods for producing ozone. One such method involves gaseous discharge type devices wherein a gas filled tube is used to produce radiation of a suitable wavelength to generate ozone. Another method is by using electrical energy from corona discharge, x-rays or cathode rays which produce radiation that generates ozone. In either case, the principle behind the production of ozone involves the oxygen bond breaking by applying energy to form a single oxygen atom, which combines with other oxygen molecules to produce ozone.

Ozone has been used in manufacturing industries such as food, beverage, pharmaceutical, healthcare, and the hospitality for almost 100 years. Even though it is known as a toxic gas but under limited range and control environment, it is used widely in industries. Many types of research have been conducted on ozone and it is found useful in microorganism treatment such as sterilisation, water treatment, food preservation, product storage, odour control, health and medicine etc. (Prabakaran, 2012; Tony, 2010;

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Hollingsworth, 2007; Azarpazhooh et al., 2008; Olmez & Akbas, 2009).

Ozone is one of the bactericidal agents which can minimise and control the bacteria activities. Ozone acts by inhibiting and blocking the operation of the enzymatic control system which leads to the destruction of bacteria. As ozone has bactericidal properties, it is used in sewage disposal plants and in meat preservation during the tenderising process (Prabakaran, 2012). In addition, ozone is used in odour control as it has a strong characteristic odour which makes it difficult to detect other odours when ozone is present. A study by Horvath, Bilitzky, & Huttner, (1985) found that ozone at a concentration of 0.01ppm can destroy microorganisms and eliminate most odours within 48 hours.

Ozone usage is popular in the food industry for food surface hygiene, sanitation of food plan equipment, reuse of waste water, and lowering biological oxygen demand (BOD) as well as chemical oxygen demand (COD) of food plant waste. Many researchers agree that ozone can be used for removing residual pesticides and maintain the freshness of perishable food like fruit and vegetable, which are easily spoiled. Generally, the storage environment is high humidity which favourable influences germicidal effect. For that reason, ozone had been used in food storage as it can oxidise and inhibit the microorganism. These properties make ozone an excellent means of increasing the shelf-life of food.

Exposure to ozone is also reported to reduce influenza disease severity and alters the distribution of influenza viral antigens in murine lungs. This is proved by the reduction of severity of disease in an animal that exposed to ozone during infection (Wolcott, Zee, & Osebold, 1982; Hollingsworth, 2007). Moreover, ozone is used in dentistry. According to Azarpazhooh and Limeback (2008), there is some evidence that shows the usage of ozone in restorative dentistry prior to etching and the placement of dental sealants and restorations.

The effects of change with ozone treatment on microorganism depend on several factors including the amount of ozone, contact time (CT), and environment condition such as temperature and pH value (Karaca and Velioglu, 2014; Huth et al., 2011; Prabakaran, Tamil, Merinal, & Panneerselvam, 2012). According to Karaca and Velioglu (2014), the efficiency of ozone decreases as the temperature of aqueous medium increases, which results in decrease solubility.

This study focuses on the development of an effective system for ozone gas and ozonated water production with different concentration and longer half-life. The existence and concentration of ozone in water were examined using Schoenbein paper. Additionally, investigation on the effect of ozone gas treatment toward the activity of microorganism was studied.

II. MATERIALS AND METHODS

A. Design and Fabrication of Ozonated Water System

Corona discharge ozone generator is selected as the main source to generate ozone gas. Corona discharge method produces ozone by passing air through a high voltage electrical discharge. Then, the energy within the discharge converts some of the oxygen to ozone as illustrated in Figure 1. The reaction can be summarised as in Equation 1:

$$3O_2 \xrightarrow{High Voltage Electricity} 2O_3$$
$$\Delta H^O = 284.6kJ \quad (1)$$

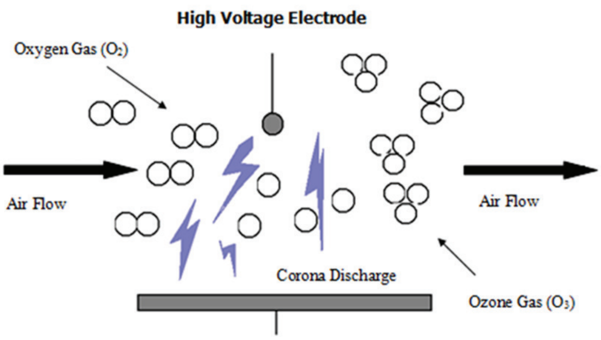


FIG. 1: Oxidation process using Corona discharge

The output rate of this system is approximately 3g/hr. Besides that, the system is air-cooled and reliable in producing ozone concentration up to 3% by weight when utilising pure oxygen. The design is equipped with a feature of ozone concentration knob to control the ozone ppm. This system can be connected to air input source of the oxygen tank, oxygen generator, internal air compressor or external air compressor. For this project, the internal air compressor was used to produce ozone.

The generated ozone air was emitted to the water through bubble diffuser or venturi injection. Firstly, the venturi injection technique as shown in Figure 2 was tested. Venturi injector (MK-684, Ozone Solution, US) works by forcing water through a conical body and creates a pressure difference between a fluid inlet and outlet ports. This action creates a vacuum inside the injector body. Then, the vacuum will allow the ozone gas from ozone generator to be added into the flowing water stream via a suction port on the injector. The ozone gas that is emitted into the injection body will diffuse into water and form ozonated water.

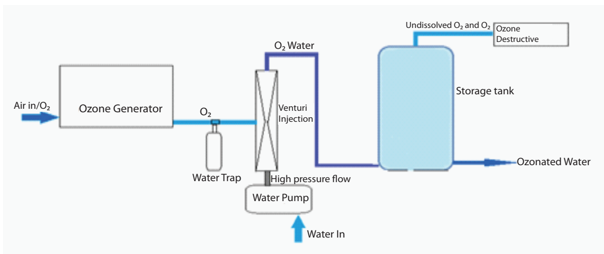


FIG. 2: Schematic diagrams of ozone water treatments using venturi injection

The second method for ozonated water production is using bubble diffusion technique as shown in Figure 3. This technique uses cylindrical diffuser stone (Ozone Solution, US) which works under pressure and creates a bubble column. The diffusers permit ozone gas from ozone generator to pass through a porous membrane thus creating many small ozone bubbles in the water. As the ozone bubble rise, the gas at the bubble's edge will be transferred into the water and ozonated water will be produced. The volume of water is an important factor in this application in order to achieve high ozone transfer efficiency.

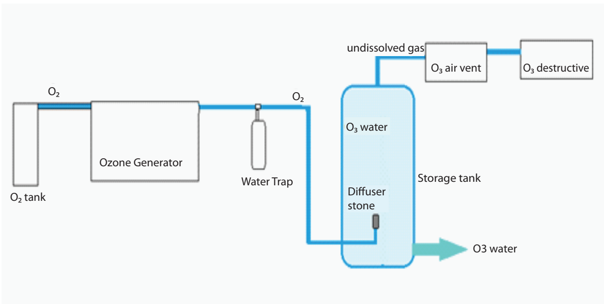


FIG. 3: Schematic diagram of ozone water treatments using bubble diffuser

For ozonated water transfer efficiency, both bubble diffuser and venturi injection technique can be used. Bubble diffuser prefers the pore size of the diffuser in order to create a smaller bubble which has greater surface area.This improves the contact between gas bubbles and water and hence increasing the mass transfer rate. Meanwhile, venturi injection method uses the water pump to increase the water pressure enter into the injector and enhances dispersion of ozone into water. High water pressure is used to produce a high concentration of ozonated water.

B. Testing the Ozonated Water Concentration

The concentration of ozonated water produced is tested using Schoenbein paper. Schoenbein paper can confirm the presence of ozone in the system. Ozone will oxidise the potassium iodide on the Schoenbein paper to produce iodine. The iodine reacts with the starch and producing the purple colour. The intensity of purple colour correlates to the amount of ozone present. The darker the colour, the more ozone is present. The reactions involved are:

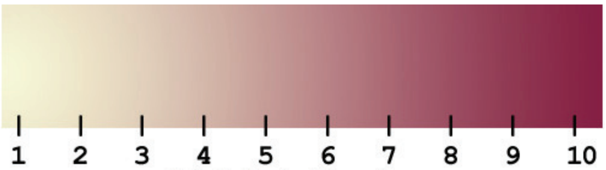
$$2KI + O_3 + H_2O = 2KOH + O_2 + 2I \qquad (2)$$

$$I + starch \rightarrow blue\ or\ purple\ colour \qquad (3)$$

The ozone reading is obtained by comparing the change of Schoenbein paper colour with the Schoenbein Colour Scale (Figure 4) and Relative Humidity Chart (Figure 5). The relative humidity for Kota Kinabalu, Sabah typically ranges from 50% to 95% over the years (WeatherSpark, 2015). In this research, the relative humidity is determined by referring Malaysia Meteorological Department (MetMalaysia, 2015) which is around 50% (mildly humid).

C. Sample Preparation and Treatment

The ozonated water produced using bubble diffuser technique was tested on the microorganism treatment using apple as the food sample.



Schoenbein Colour Scale	
0 - 3	Little to no change
4 - 6	Lavender hue
7 - 10	Blue or purple

FIG. 4: The schoenbein colour scale

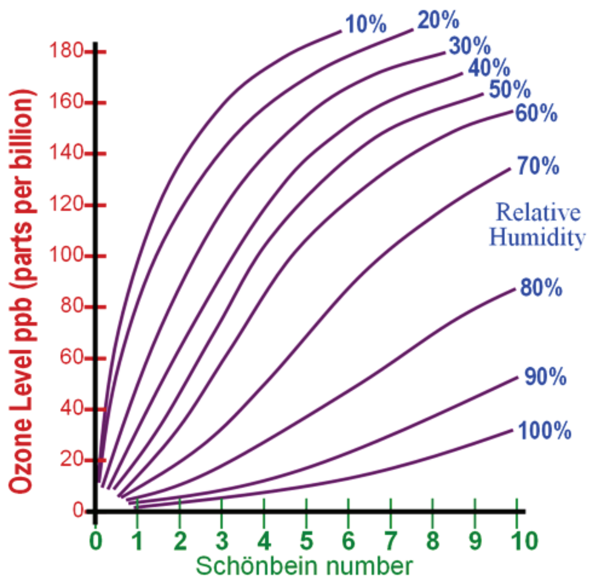


FIG. 5: Relative Humidity Schoenbein

The results were compared with another two different treatments using tap and distilled water. The media was prepared to culture the microorganism. The microbe sample from each treated apple was smeared on an agar plate and incubated for 24 hours. Then, the colonies of the microorganism were calculated by counting each colony dot that appeared on the media (Putman, Burton, & Nahm, 2005).

D. Media Preparation

500ml distilled water and 10g sodium agar were placed into the 500ml beaker. The mixture was stirred until all was dissolved. The mixture was then poured into Scott bottle covered with aluminium foil. Then, the bottle was autoclaved for two hours. Next, the mixture was poured into the petri dish in the laminar flow cabinet that had been sterilised with 75% ethanol. The petri dish was sealed with parafilm and was placed in the clean area for 24 hours.

E. Culturing Microorganism on the Surface of Apples

Four apples were prepared for this experiment. For the first condition, the apple was washed with tap water containing chlorine. As for the second condition, the apple was washed with distilled water. For the third condition, the apple was washed with ozonated water which was produced using bubble diffuser technique. The fourth apple was not washed and it was taken as the control sample. After that, the apples were placed inside the sterilised laminar flow. The surfaces of each treated apples were smeared to the media. The Petri dishes were left for 24 hours in the incubator at temperature 30°C for microorganism growth on the media.

F. Estimating the Ozonated Water Concentration

The needed rate of ozone production in achieving a specific concentration of ozone gas into a water flow is determined using the formula below:

$$water\ flowrate \times O_3\ concentration = required\ rate\ of\ O_3\ production \quad (4)$$

To obtain 20 ppm of ozone, the rate of ozone gas produced is 90g/hr. However, this does not mean that the ozone will be fully dissolved due to efficiency losses with injecting ozone and ozone demand of the water.

G. Determination of Ozone Output using Oxygen Feed Gas

The output of ozone generator using oxygen feed gas is determined. The standardised flow rate of oxygen and the concentration of ozone are required to calculate the production rate of ozone generator using Equation 4:

$$O_2\ flowrate \times O_3\ concentration = O_3\ production\ rate \quad (5)$$

where the rate has the dimension of $\frac{mg}{hr}$.

H. Statistical analysis

All experiments were repeated five times. The t-test is applied when there is a necessity to assess the means of two group results at two variables is statistically different from each other (Celikyilmaz and Türksen, 2009). Thus, the t-test is carried out in this research to assess the significance of the difference between the various treatments on the fruit sample.

III. RESULTS AND DISCUSSION

A. Schoenbein test

The Schoenbein test is conducted by testing the Schoenbein paper with two type of ozonated water as a product of two different working principles which is venturi injection and bubble diffusion. Table 1 and Table 2 show the result of the Schoenbein paper after being sprayed with the ozonated water. During the experiment, the ozonated water was applied to all part of Schoenbein paper for the duration of ±30 seconds to monitor the colour changing.

TABLE I: Results of Schoenbein papers test for venturi injection technique

Sample	Schoenbein paper	Schoenbein colour scale	Estimated of relative humidity (%)	Estimated of ozonated water concentration (ppm)
1		2	50	0.05
2		2	50	0.05
3		2	50	0.05
Average estimation of ozonated water concentration				0.05

TABLE II: Results of Schoenbein papers test for bubble diffuser technique

Sample	Schoenbein paper	Schoenbein colour scale	Estimated of relative humidity (%)	Estimated of ozonated water concentration (ppm)
1		4	50	0.10
2		4	50	0.10
3		3.5	50	0.09
Average estimation of ozonated water concentration				0.097

Referring to the Table 1 and Table 2, the colour change of bubble diffuser technique is darker than venturi injection technique. The Schoenbein colour scale for venturi injection is 2 and for bubble diffuser around 3.5 to 4. Furthermore, the average estimation of ozonated water concentration for venturi injection technique is 0.05 ppm while for bubble diffuser technique is 0.097 ppm. Based on this result, it shows that the bubble diffuser technique can produce a higher concentration of ozonated water than venturi injection, which also means that higher ozone transfer efficiency can be achieved using this technique. However, from the previous literature venturi injection shows a higher ozone transfer rate compared to bubble diffuser (Rip and Mark, 2011). Hence, the results shown in this project are different from the previous findings due to the absence of contact tank in this system. The presence of contact tank is important to allow additional time for more ozone to dissolve in the water. The dissolved ozone in this medium will oxidise minerals and disinfect the water. Therefore, when the venturi injection is used with contact tank, the concentration of ozonated water produced will be higher.

B. Activity of Microorganisms after Treatment

Ozone is one of the antimicrobial agents which can minimise and control the microorganism activities. Microorganisms are very small and its body is sealed by a relatively solid cell membrane. Yet, their vital processes are controlled by a complex enzymatic system. Ozone act by inhibiting and blocking the operation of an enzymatic control system which leads to the destruction of microbial (Prabakaran, 2012).

The activity of microorganism is related to the growth rate of microorganism. Through this experiment, the growth rate of microorganism is calculated in term of colony dot after been treated with three different methods which are tap water, distilled water and ozone water. Referring to Figure 6, the growth of microorganism using ozonated water treatment shows the lowest colony with the average of 2.50. Colonies of microorganism are significantly lower when treated with ozonated water ($p < 0.01$). This proves that the ozonated water is the most effective method to kill microorganism compared to the other two methods.

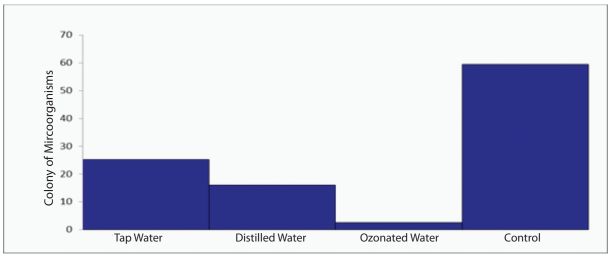


FIG. 6: Colony of microorganism after each treatment

C. Half-life Enhancement of Ozonated Water

The relation between storage temperature and half-life of ozonated water is as shown in Figure 7. The half-life of ozonated water is approximately 50 minutes for 10°C. When the temperature becomes higher which at 30°C, the half-life of ozonated water is lower which around 12 minutes. There is a significant positive relation between the temperature of ozonated water and half-life ($p < 0.05$).Therefore, maintaining the ozonated water at a lower temperature is important in order to maintain the longer half-life of ozone in water.

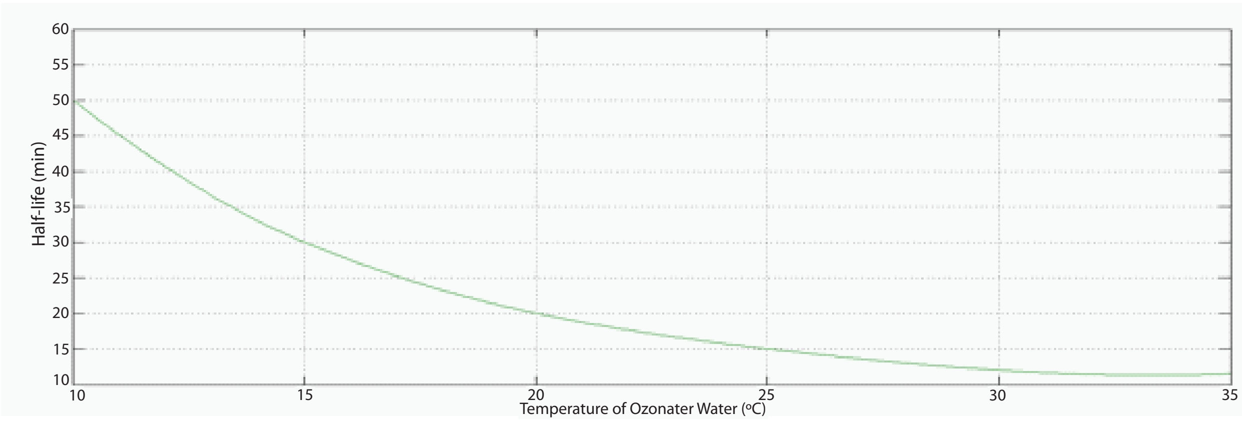


FIG. 7: The half-life of ozonated water as a function of temperature

IV. CONCLUSION

The experimental findings prove that the ozonated water treatment using bubble diffuser technique shows better efficiency in microorganism treatment when compared with tap water and distilled water treatments. Furthermore, the study found that bubble diffuser technique can produce a higher concentration of ozonated water than venturi injection technique due to the absence of contact tank in this system. This shows that contact tank is needed in the ozonated water system in order to increase the percentage and probability of ozone diffusion into the water. The half-life of the ozonated water can be improved by decreasing the storage temperature and also by increasing the collisional rate between the ozone gas bubbles and water.

V. ACKNOWLEDGEMENT

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