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Article

Effectiveness Evaluation for Bacteria and Fungi Disinfections of Developed Ozone Machine

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Abstract. A low-cost ozone machine was developed to generate gaseous ozone for decontaminating the non-ventilated and unoccupied room within a 50-minute ozonation duration. This ozone machine, equipped with a centrifugal blower, could assist in thoroughly dispersing ozone gas throughout an enclosed area, as a result this ozone machine could raise the ozone concentration up to 2.4 ppm inside a 50 square-meter classroom after the ozonation period. Performances of this ozone machine were experimentally evaluated inside both non-ventilated and ventilated classrooms. First, an average total ozone dose of 58 ppm-min, which was close to an upper limit for the >90% viral disinfection, demonstrated the machine effectiveness in the viral inactivation. Second, a waiting period for ozone decomposition and removal using a ceiling ventilated fan could be predicted from an exponential decay rate of ozone concentration to be about 1.5 hour before the decontaminated room could be re-entered safely. Third, the disinfection of bacteria and fungi, which are tougher than most viruses, were validated by the cultural tests using standard plate count agars after the 50-minute ozone exposure. Results of the colony count and microorganism identification using the MALDI-TOF MS and the fungi slide culture technique revealed that 11 species of bacteria and 5 species of fungi could be inactivated by ozone gas after the specified ozonation period.

Keywords: Ozonation, total ozone dose, ozone decomposition, fungi and bacteria disinfection, cultural test.

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1. Introduction

To prevent a spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) in school, restaurant, hospital, and public transportation, several standards: wearing surgical mask, cleaning hand, and physical distancing have been established during pandemic. The SARS-CoV2 can spread via airborne particles and droplets as well as via contacting with respiratory fluids, containing the infectious virus and settling down on different surfaces. Infected particles can transmit disease up to a distance of 6-feet and stay airborne for few hours in indoor environments [1]. Thus, to disinfected enclosed areas from airborne viruses, including influenza, rhinovirus, and SARS-CoV-2 virus, and to eradicate pathogens on contaminated fomites, various existing sterilizations have been applied in many researches; for example, 1) chemical-base disinfection using sodium hypochlorite solution, alcohol- and quaternary ammonium compound-base agents, 2) ultraviolet irradiation, and 3) ozonization. The UV irradiation, used UV-C light with wavelength of around 254 nm, can disinfect wide ranges of microorganisms by damaging cell DNA and killing living organisms [2]. Even though, the UV approach is low-cost and no chemical residue, UV-C light might not be able to thoroughly expose to all decontaminated surfaces where shadow of objects blocks the UV irradiation in vast public areas. Moreover, the UV-C sterilization process must be performed in unoccupied room due to its hazard to human health; such as risk of skin burn and cancer or blindness. The sodium hypochlorite (>0.21%), ethanol (>75%), and hydrogen peroxide (H_2O_2 > 0.5%) are among common chemical-base disinfectants [3], used in the large-scale inactivation of viruses, especially SARS-CoV2 and influenza. Ethanol with 70% concentration can disinfect CoVs within 30 seconds of evaporation period. The hydrogen peroxide of 1-3% concentration can disinfect CoVs within 1 min by lipid oxidation and denaturation of CoVs and membrane destruction. Because of corrosive property of the sodium hypochlorite, disinfection process might cause irritation of skin and airway membranes [6]. However, the limitations of chemical-base disinfectants for room decontamination are that chemical aerosol must be sprayed onto contaminated surfaces and rub/clean thoroughly for a certain period, which is time consuming.

Ozone, consisting of three oxygen atoms (O_3), is a highly oxidative substance that can be used for disinfecting pathogenic microorganisms; like, CoVs, bacteria, and fungi in both phases: gaseous ozone and aqueous ozone. Because of ozone's strong reactive characteristics, ozone has been applied to various industries: 1) food industry for bacteria sanitization [4], aflatoxin-contamination reduction [5], 2) healthcare for medical equipment sterilization [6], disinfection of pathogenic bacteria with antimicrobial resistance [7, 8] and disinfection of SARS-CoV2 [13, 14], 3) garment industry for bacteria and fungi rapid inactivation [9], 4) water sanitization for water treatment in aquaculture system [10] and water purification [11], as well as 5) storage industry for pest control in stored products [12]. According to its oxidant capability, lipid envelop of CoVs can be broken apart, leading to virucidal effect. Moreover, ozone can destroy bacterial and fungal cell wall and cell membrane resulting in spores and nucleic acid inactivation [4, 8, 25]. Furthermore, major advantages of ozone gas over the chemical-base disinfection are that it is less expensive than sodium hypochlorite and alcohol in terms of large-area disinfection and it does not leave any residues after the disinfection process. Since ozone gas is unstable, ozone naturally and gradually decomposes into oxygen. However, due to toxicity of gaseous ozone to human health, ozone disinfection is limited to perform only in unoccupied and sealed rooms [13]. In addition, a waiting period after ozonation must be determined from ozone decomposition rate before safely reusing sanitized areas.

At ambient temperature, a gaseous ozone density of 2.14 kg/m^3 is higher than air density of 1.20 kg/m^3. As a result, ozone gas eventually subsides close to the ground floor during the disinfection process, thus ozone can disinfect both airborne and direct-contact viruses, including SARS-CoV2, in confined areas; such as, hospitals, schools, universities, and et. al. To disperse ozone gas inside the enclose area, ozone machines are often equipped with a fan or air jet so that difficult-to-clean region/surfaces can be reached and decontaminated by ozone gas. According to Table 1, the developed ozone machine is compared with commercial ozone machines in terms of an amount of generated ozone gas, a maximum volumetric-flow capability, a suitable room size, and price. Therefore, an appropriate flow stream from the circulation fan must be properly designed and chosen to ensure a uniformly distribution of gaseous ozone inside the room. However, most the commercial ozone machines lack a validation or certification of the virus or bacterial disinfection, which are shown in this paper for the developed ozone machine.

Table 1. Comparison of commercial ozone machines on the market with the developed low-cost ozone machine.

Ozone Machine	Ozone Generation [mg/h]	Max. Flow Capability [m^3/h]	Room Size [m^2]	Price [bath]
AIKO HE-150R machine	4,000	3.0	Not specified	3,690
Mazuma T-1000P machine	1,000	Not specified	250	12,900
Airwave D-250 machine	20,000	Not specified	30-50	9,800
Developed machine	1,500	2.3	50	2,840

First, the disinfection mechanism as well as the desired viral disinfection condition using ozone gas are

described in Section 2. Furthermore, health concern in terms of safe level of ozone concentration must be aware and ozone decomposition can be estimated from its half-life time before users can enter the decontaminated room safely. Second, the developed ozone machine along with air-flow analysis for distributing ozone gas inside the enclosed and unoccupied room are discussed in Section 3. In addition, the viral inactivation in classroom is validated according to amount of the total ozone dose and the safety period after ozone decomposition is estimated from the half-life time of ozone gas, derived from experimental results. Lastly, Section 4 demonstrates the effectiveness of this developed ozone machine to inactivated both bacteria and fungi, which are more ozone-tolerant than virus due to their thicker cell wall. The cultural test using a standard plate count revealed that oxidation process of ozone gas could inactivate several species of bacteria and fungi.

2. Ozone Disinfection Mechanism, Health Safety and Ozone Generator

2.1. Disinfection Mechanism of Ozone

Mechanism for microorganism inactivation with ozone gas is based on breaking apart the envelope protein of living-cell molecules with either single or multiple bonds by ozone's oxidative action as well as damaging the nucleic acid core [14]. The efficacy of ozone gas disinfection depends on several parameters: ozone concentration, contact time, temperature and relative humidity of ambient air. Several researches on ozone disinfection for airborne viruses and bacteria employed low ozone concentration: 0.5-5 ppm for 30-minute exposure time [15], and 0.23-1.23 ppm for 70-minute exposure time [13]. On the other hand, high ozone concentration of 20-25 ppm could inactivate 12 different viruses within a 15-minute exposure period [16]. Therefore, a product of ozone concentration (OC) in ppm and exposure time in minute yields a "total ozone dose" [14, 17], that can be quantified as a main parameter for 90% viral inactivation, as shown in Fig. 1. The total ozone doses of 20-112 min·mg/m^3 (or 10.19-57.05 min·ppm) and 47-223 min·mg/m^3 (or 23.94-113.59 min·ppm) require for >90% viral disinfection and for >99% viral disinfection, respectively. The >90% Viral Disinfection Time (VDT) in minute [14] can be predicted from the amount of ozone concentration in Eq. (1), shown in Fig. 1. Several researches [13, 18] reported a strong correlation in a feasibility of the viral inactivation using ozone gas with the relative humidity and exposure time. The higher relative humidity of air was, the more powerful ozone disinfection of viruses became.

$$VDT = -24.18\ln(OC) + 77.10 \quad (1)$$

2.2. Safety and Health Concern

Due to highly reactive properties of ozone gas, the Immediately Dangerous to Life or Health (IDLH) Concentration value was declared by National Institute of Occupational Safety and Health (NIOSH) that ozone concentration of 5 ppm and above can cause acute inhalation toxication in human [19]. Also, the NIOSH recommends that an upper limit for the ozone concentration of 0.1 ppm should not be exceeded at any given time [20]. Potential risks of exposing to the ozone concentration of more than 0.1 ppm for long duration can cause throat irritation, decreasing in lung function, chest pain, aggravation of asthma, and et. al. [18, 20]. Nevertheless, instable ozone gas can be naturally decomposed into diatomic oxygen with an average Half-Life Time (HLT) of about 1,500 minutes in an ideal condition: still air, zero humidity, and temperature of 24℃. According to experimental tests in [21], the HLT of ozone gas inside an enclosed plexiglass cylinder for the ideal condition depends on three main parameters: 1) initial ozone concentration in ppm (*IC*), 2) temperature in ℃ (*T*), and 3) relative humidity in % (*RH*). The Eq. (2), expressed the ozone HLT in minute, can be employed as an upper-bound limit for the ideal ozone decomposition without any surrounding interaction.

$$HLT = 2274.4 + (0.483 \cdot IC) - (51.64 \cdot T) - (12.01 \cdot RH) \quad (2)$$

However, the ozone HLT for an indoor condition can vary within 7-10 minutes [22]. An outdoor ozone concentration, an air-exchange rate through ventilation systems, surface-removal rates of different materials inside indoor environment, and reactions between gaseous ozone and other chemical compounds in the air can have a direct influence on the ozone HLT.

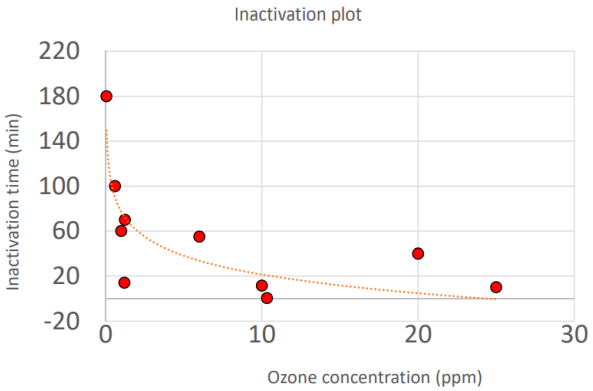

Fig. 1. Total ozone dose: a product of ozone concentration and exposure time was summarized from several researches for 90% viral inactivation [14].

2.3. Ozone generator using corona discharge

An in-situ generation of the gaseous ozone from air or oxygen gas is generally performed inside the ozone generator machines. There are several types of ozone generators; such as, 1) photochemical method using ultraviolet irradiation, known as the UV-ozone, and 2) dielectric barrier discharge (DBD), which is an inexpensive device used in this research, displayed in Fig. 2. Corona discharge of the DBD occurs when strong electric field ionizes surrounding air/oxygen without generating any arc between two electrodes. In high-voltage systems; like power transmission line or high-voltage

transformer, the corona effect, producing a hissing or crackling sound, can be developed unless proper precautions are taken to minimize an intensity of the surrounding electric field.

The ozone generator of DBD type used in this ozone machine can produce gaseous ozone with a rate of 1,500 mg/h. Therefore, to disinfect a 50 square-meter classroom with 3.0-m height, a maximum ozone concentration of 8.33 mg/m^3 can be generated within 50 minutes, which can be converted into parts per million (ppm) unit [14], as expressed by Eq. (3).

$$8.33 \, {}^{mg}\!/_{m^3} \cdot \left({}^{24.45}\!/_{48} \right) = 4.24 \, ppm \qquad (3)$$

where a value of 24.45 denotes a volume of 1 mole of an ideal gas at atmospheric pressure of 1 atm and temperature of 25 ℃ and a molecular weight of ozone gas is 48 g/mol.

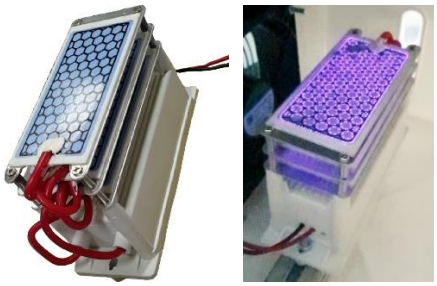

Fig. 2. Ozone generator of the Dielectric Barrier Discharge (DBD) type can produce ozone gas from the corona discharge.

3. Ozone Sterilization Machine

Both the effectiveness of virus/bacteria sterilization and a safety of user health were main objectives for this low-cost ozone-machine development. Furthermore, the ozone machine was designed and constructed for sterilizing contaminated and unoccupied classrooms or meeting rooms of size up to 50 square meter using gaseous ozone. Uniform dispersion of ozone gas could be accomplished with a centrifugal blower that could generate high flow rate so that low ozone dose could be deployed to cover vast surfaces inside the enclosed area. The higher the flow rate was, the more the ozone gas could be distributed in a classroom of 150 cubic meters at a concentration of at least 1–2.5 ppm over a 50 minutes period.

3.1. Machine Components

The developed ozone machine was modified from a light-weight misting-fan machine that was equipped with a centrifugal blower, as shown in Fig. 3. The blower with 3-speed level could help carrying ozone gas to farther distance of about 5 meters, thus ozone gas could be circulated thoroughly within the closed and unoccupied room. For a safe operation of users, a timer function for turning on/off each ozone machine was implemented with an electronic circuit, displayed in Fig. 3, consisting of 1) an Arduino MEGA microcontroller, 2) two push buttons for setting a delay time, 3) a toggle switch for counting down the delay time, 4) a relay board for electronically turning on/off the ozone generator, and 5) an LCD screen for informing machine status to users. Push buttons could increment or decrement the delay time by 10 minutes each time button was pressed. The maximum delay time of 3 hours could be set by the user. From Eq. (1), the viral inactivation period for Ozone Concentration (OC) of 4.24 ppm, derived from Eq. (3), could be estimated to be 42.14 minutes, therefore the sterilizing time of this developed ozone machine was programmed to turn-on for 50 minutes.

Fig. 3. The electronic circuit on top of this developed ozone machine (left) and the centrifugal blower inside this ozone machine (right).

3.2. Air-Flow Analysis of Developed Ozone Machine

To determine the flow characteristics of this ozone machine, air-flow simulations were performed and compared against air-velocity measurements using a hot-wire anemometer. The air-flow simulations of the centrifugal blower, rotating at angular speed of 1,200 rpm, were accomplished with the SolidWORKs flow simulation. An initial air velocity inside a closed 50-m^2 room was set to be zero and the blower height was positioned at 0.45 meter from the ground, the same height as the developed ozone machine, as shown in Fig. 4. A rectangular channel of a 60-cm length, covering an exit of the centrifugal blower, was constructed inside the SolidWORKs flow simulation to obtain a steady-flow condition. The air-flow simulation revealed that still air from a side of blower was swirly drawn into an inlet wheel and pushed out through blower impellers, as shown in Fig. 5.

Fig. 4. The ozone machine was developed in this research (Left) and this ozone machine was equipped with a rectangular air-flow channel for measuring steady air speed from the centrifugal blower (Right).

Air-flow velocities at various distances were measured by a testo-435 flow meter at several heights from the

channel top, as illustrated in Fig. 6. These measurements were then compared against the air-flow simulations in case of no external flow disturbance. According to results of flow simulations and measurements in Table 2, the air velocities in the middle of channel (or 9-cm down from the top of channel) were closely matched at short distances of 10-cm and 20-cm away from the blower exit. However, at a further distance of 50-cm from the blower exit, the simulated velocities became higher than those from the measurement, especially near the top region of the channel. Even though, the steady flow in the flow simulation could be assumed, but a viscosity effect dominated near wall boundaries, which significantly subsided the measured air-flow velocity. One of the main reasons causing the velocity at the channel top to be higher in both simulation and measurement in Table 2 was that the further radial distance of the impeller blade tip created the larger momentum of air flow. Furthermore, to validate flow distance that could be achieved by this blower in wide forward direction, flow of smoke from incent sticks were experimentally observed up to 5 meters away from the ozone machine, as shown in Fig. 7.

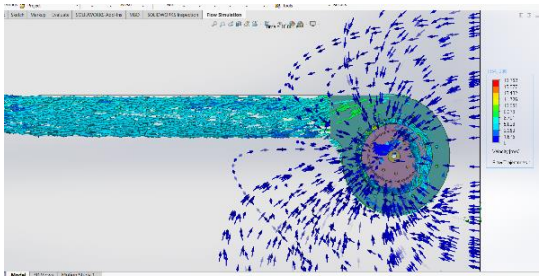

Fig. 5. The simulation of air-flow velocities inside the rectangular channel, directly connected with the centrifugal-blower exit, was performed by the SolidWORKs flow simulation.

Fig. 6. Measurements of air-flow velocities using the hot-wire anemometer were performed inside a rectangular channel connected to an exit of the centrifugal blower of the developed ozone machine.

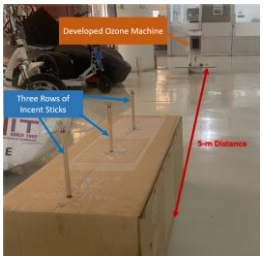

Fig. 7. Air flow could be observed at 5 meters away from the ozone-machine blower exit in open environment.

Table 2. Air-flow velocities from the centrifugal blower.

Distance from blower exit (cm)	Distance from the channel top (cm)	Measured Air Velocity (m/s)	Simulated Air Velocity (m/s)
10	9	6.0 – 7.0	6.6-7.0
20	9	2.5 – 3.0	2.7-3.5
	6	6.0 – 7.0	6.6-7.0
50	9	1.5 – 2.0	2.2-3.2
	6	1.8 - 2.3	2.8-3.5
	5	3.1 - 3.6	3.8-4.5
	4	4.3 – 4.5	4.8-5.9
	3	4.7 – 4.9	6.2-6.8

3.3. Ozone Concentration/Dose during Classroom Disinfection

The disinfection processes were performed and evaluated in a closed and unoccupied 50-m^2 classroom without any air circulation from an air condition, a ceiling fan, or natural convection through open windows. Ozone concentration inside the classroom was measured using two PONPE 311-O3 ozone detectors, that could measure O_3 within a range between 0 and 20 ppm. The detector1 was placed in the middle of classroom and the detector2 was positioned at the opposite classroom corner from the ozone machine, which were correspondingly 4 and 8 meters away along a straight path from the ozone machine's exit. Both ozone detectors were located on lecture chairs with foldable table top at a height of 77 cm from the floor. During the 50-minute disinfection period, the Ozone Concentration (OC) at locations of two specified detectors was monitored and recorded by two laptops, as shown in Fig. 8. Figure 9 shows a measurement of ambient temperature and Relative Humidity (RH) inside the room by a DHT11 sensor. According to in Fig. 10, the ozone concentration increased and concaved down as a parabolic function of time when the ozone gas was generated and accumulated within the closed room. The RH significantly influenced the amount of generated ozone concentration, while the ambient temperature had a minor effect on the ozone concentration. When the RH becomes large, the maximum ozone concentration, that could be generated inside the classroom, was reduced, as shown in Fig. 11.

Fig. 8. Ozonation experiments were performed inside the 50-m^2 classroom. Using two ozone detectors at location 1 and 2, OC, ambient temperature and RH could be monitored and recorded during the 50-min period.

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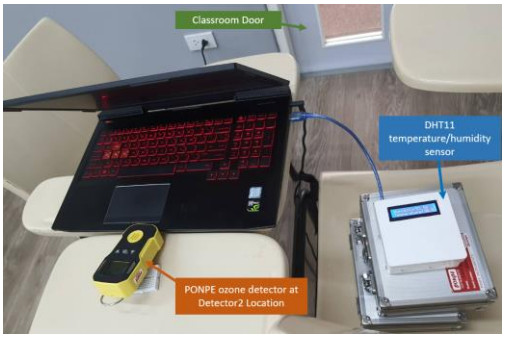

Fig. 9. PONPE ozone detector at the detector2 location and the DHT11 sensor were employed for measuring ozone concentration and ambient temperature/humidity, respectively.

To ensure the >90% viral disinfection, the Total Ozone Dose (TOD) could be calculated from an area under the graph of Ozone Concentration (OC), measured by both ozone detectors in Fig. 10. In Table 3, the average TOD were derived from three experimental tests inside the 50-m² non-ventilated classroom from three different days with varying temperature and relative humidity. These average TOD were close to an upper limit of 57.05 min·ppm for the >90% viral disinfection range and were much higher the lower limit of 23.94 min·ppm for the >99% viral disinfection range [17]. As a result, the TOD could assure an effectiveness of the >90% viral disinfection using this developed ozone machine.

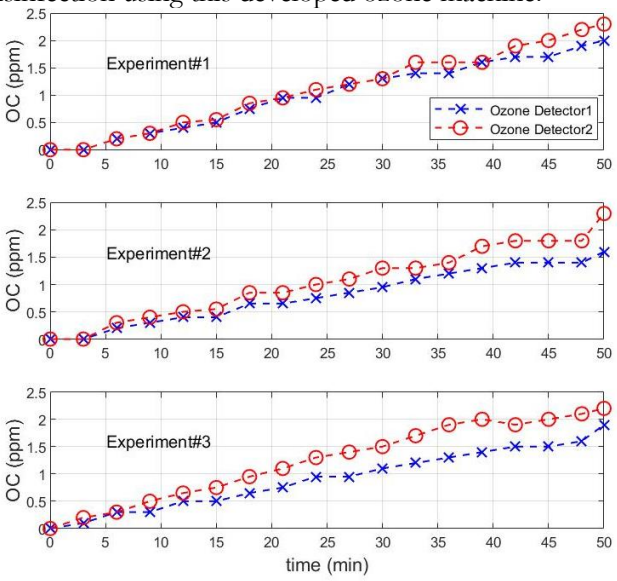

Fig. 10. For 3 experimental tests inside a non-ventilated classroom, ozone concentrations were measured by ozone detector1 (blue cross line) and detector2 (red circle line) during the 50-min turning-on period of the ozone machine.

An effect of the room ventilation using a ceiling fan on OC was investigated in Fig. 12. The Total Ozone Doses (TOD's) at both ozone-detector locations did not increase as much as the previous cases without ventilation during the 50-min ozonation process. Thus, the TOD's values shifted toward a lower limit of the >99% viral disinfection range. These results confirmed that the sterilization process would be more effective for viral disinfection, if it performed within the non-ventilated room where the ceiling fan, windows, and the air condition were closed. In addition, low relative-humidity condition of the ambient air also had a strong influence on OC and TOD, as demonstrated by both experiment#1 results in Fig. 10 for the non-ventilated room and in Fig. 12 for the ventilated room.

Table 3. Total Ozone Dose (TOD) were computed from the area under the graph of Fig. 10. Test conditions: temperature (T) and relative humidity (RH) are expressed by mean ± standard deviation, from 3 different experiments inside the 50-m² non-ventilated classroom.

Test No.	TOD1 [ppm-min]	TOD2 [ppm-min]	T_{avg} [°C]	RH_{avg} [%]
1	51.75	57.00	29.54±0.56	57.93±1.75
2	41.25	53.40	29.89±0.66	64.60±2.34
3	46.65	64.05	32.01±0.14	64.10±0.28

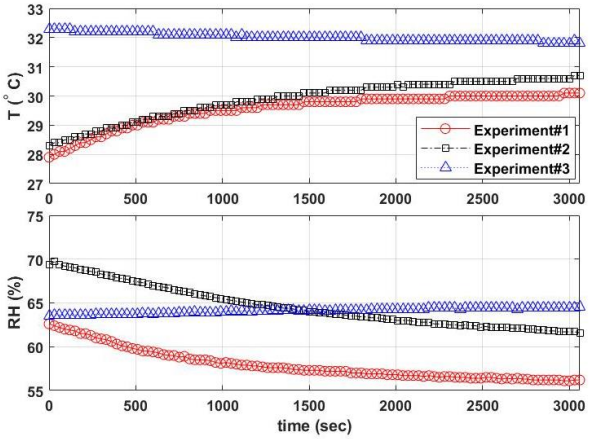

Fig. 11. Temperature (Top) and relative humidity (Bottom) were measured during the 50-min turning-on period of the ozone machine inside the 50-m² non-ventilated classroom from 3 different experiments.

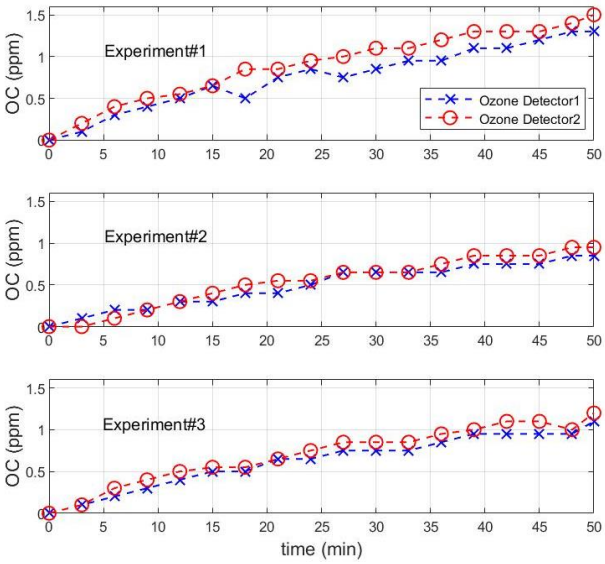

Fig. 12. For 3 experimental tests inside ventilated classroom, ozone concentrations were measured by ozone detector1 (blue cross line) and detector2 (red circle line) during the 50-min turning-on period of the ozone machine.

Table 4. Total Ozone Dose (TOD) were derived from the area under the graph of Fig. 12. Test conditions: temperature (T) and relative humidity (RH) are expressed by mean ± standard deviation, from 3 different experiments inside the 50-m² ventilated classroom.

Test No.	TOD1 [ppm-min]	TOD2 [ppm-min]	T_{avg} [°C]	RH_{avg} [%]
1	38.70	46.20	30.18±0.82	60.47±2.60
2	25.58	27.83	30.35±0.64	66.32±1.85
3	32.25	36.30	29.52±0.98	68.20±2.76

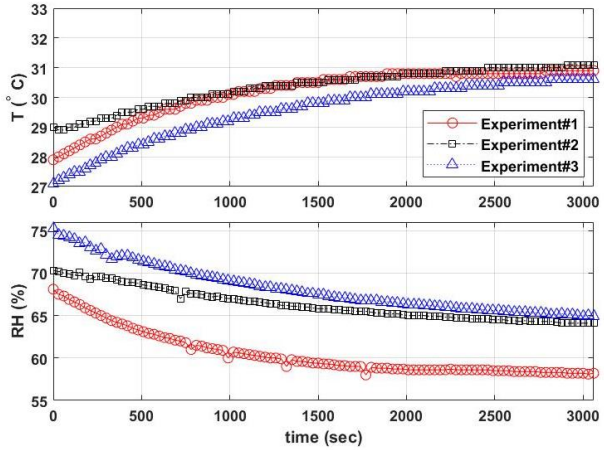

Fig. 13. Temperature (Top) and relative humidity (Bottom) were measured during the 50-min turning-on period of the ozone machine inside the 50-m² ventilated classroom from 3 different experiments.

For predicting ideal Half-Life Time (HLT) in a closed and non-ventilated classroom using Eq. (2), the maximum ozone concentration after the disinfection period of 50 min was used as an initial concentration value along with the average temperature (T_{avg}) and average relative humidity (RH_{avg}), given in Table 3 and 4. The upper bounds or ideal limits of HLTs at the ozone detector2 position for three experimental tests inside the non-ventilated room were 714.40 min, 682.76 min, and 652.56 min, respectively. Likewise, the ideal HLTs at the ozone detector2 position inside the ventilated room were 709.36 min, 699.62 min, and 742.38 min, respectively. On the other hand, to estimate the HLT of the ozone decomposition from experimental measurements in Fig. 14 and 15, a regression method using an exponential function with a percent decay rate (r), expressed in Eq. (4), could be fitted with three experimental results of both non-ventilated and ventilated conditions in a least-square sense.

$$OC = OC_{init} e^{-r \cdot t} \quad (4)$$

where OC_{init} was denoted an initial ozone concentration inside the classroom after turning off the ozone machine.

The HLT from experimental tests could be estimated from Eq. (4) when $OC = OC_{init}/2$ and t = HLT. As a result, the estimated HLT (HLT_{est}) could be computed from Eq. (5).

$$HLT_{est} = -\ln(0.5)/r \quad (5)$$

Table 5. Exponential Decay rate and estimated Half-Life Time (HLT) at positions of ozone detector 1 and 2 were computed from 3 tests using the exponential regression inside the 50-m² non-ventilated classroom.

Test No.	Detector1 Decay Rate (r_1) [1/min]	Detector2 Decay Rate (r_2) [1/min]	HLT_{est1} [min]	HLT_{est2} [min]
1	0.0101	0.0122	68.46	56.77
2	0.0100	0.0133	69.34	52.16
3	0.0112	0.0151	61.67	45.97

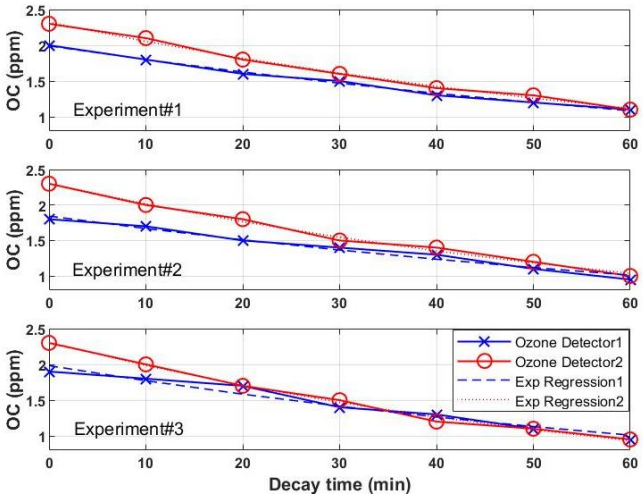

Fig. 14. Three experimental tests inside the 50-m² non-ventilated classroom, ozone-concentration decay rates were measured from ozone detector1 (blue cross line) and detector2 (red circle line) during the 60-min duration after turning off the ozone machine.

From Table 5, average values of the estimated HLT from Eq. (5) at detector1 and detector2 positions inside the non-ventilated room were 66.49 and 51.61 minutes, respectively. The ceiling fan could help reducing the average HLT in the ventilated-room case down to 23.43 and 16.57 minutes at corresponding detector1 and detector2 locations in Table 6, therefore the ventilation could significantly reduce the estimated HLTs at all locations up to 3 times. Hence, the ceiling fan should be opened after the ozone disinfection process to accelerate the ozone decomposition to be below 0.1 ppm within 1.5 hours before the decontaminated room could be entered safely. Additionally, natural convection to further quickly dimmish the ozone concentration could be achieved by opening all windows after the 50-min ozonation duration. However, the upper bound limits of HLT in the ideal condition were much higher than those HLTs, predicted by the exponential regression from all experimental results. Nevertheless, these upper bound limits of HLT remained within the same range of the ozone indoors HLT [22] because of several factors: 1) indoor air exchange with outdoor air through ceiling ventilation and gaps under two classroom doors as well as 2) surface removal rate of different materials within the classroom. Specially for the experiment#3, the high RH condition could induce larger deposition velocity of ozone onto various surfaces. Moreover, the percent decay rate at the detector2 position

was larger than that at the detector1 position due to a higher air-exchange rate near the classroom door.

Table 6. Exponential Decay rate and estimated Half-Life Time (HLT_{est}) at positions of ozone detector 1 and 2 were computed from 3 tests using the exponential regression inside the 50-m^2 ventilated classroom.

Test No.	Detector1 Decay Rate (r_1) [1/min]	Detector2 Decay Rate (r_2) [1/min]	HLT_{est1} [min]	HLT_{est2} [min]
1	0.0247	0.0368	28.01	18.81
2	0.0308	0.0438	22.51	15.83
3	0.0351	0.0460	19.77	15.06

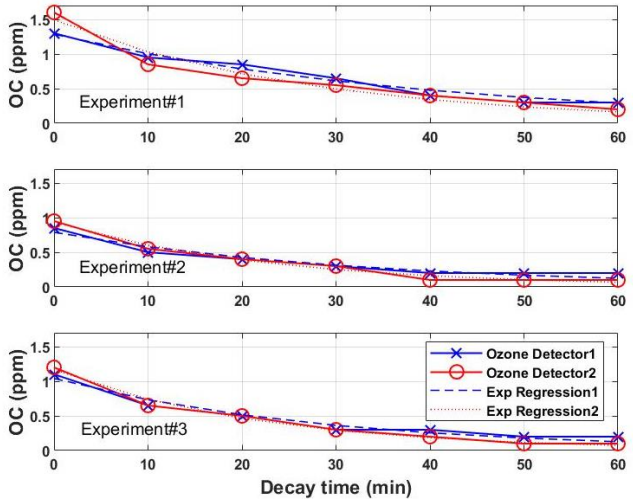

Fig. 15. Three experimental tests inside the 50-m^2 ventilated classroom, ozone-concentration decay rates were measured from ozone detector1 (blue cross line) and detector2 (red circle line) during the 60-min duration after turning off the ozone machine.

4. Bacteria/Fungus Disinfection Tests with Ozone Machine

To evaluate the effectiveness of bacteria/fungi inactivation using this developed ozone machine, bacteria/fungus culture tests were performed inside a closed room without any ventilation from the ceiling fan and the air condition inside the Thammasat University Molecular Innovation Research Laboratory (TUMIRL). Figure 16 displays a meeting room with a dimension of 6.2x7.8x2.6 m, similar size to the test classroom in Section 3, where ozonation processes were carried out. Before performing disinfection process, two plates of open-lid sterilized standard Plate Count Agar (PCA) were placed at multiple locations with various heights and radial distance from the developed ozone machine at a room temperature of 25°C for 30 min, which these sets were used as controlled reference for each location. After the room was exposed to ozone gas for 50 minutes and left for 2 hours without any ventilation, another two duplicated PCA plates were placed and processed in the same way as the control references. All PCAs were collected and incubated for 3 days at 35°C. Colony count on PCA plates was done and microorganisms were identified using the MALDI-TOF mass spectrometry (MS) for bacteria [23] and the single slide culture technique for fungi [24].

Results of bacteria and fungi culture tests in Fig. 17, 18 and 19, revealed that after 50-minute ozone exposure, ozone concentration within a range of 3-4 ppm could effectively eliminate both bacteria and fungi within the non-ventilated meeting room comparing to the controlled PCA plates without exposing to the gaseous ozone at the same locations. Only the ozone detector 1 was placed on the meeting table, 72-cm above the floor, for monitoring the ozone concentration during the disinfection tests. PCA plates at both position 1 and 2 in Fig. 16 were located within a 5-meter radial distance and about the same height in forward area of the blower, thus ozone gas can effectively inactivate bacteria/fungi at these two positions. Even though, position 4 on the coffee table and position 6 on the high shelf were located at the opposite corner of the room from the ozone machine, the bacterial and fungus inactivation by the gaseous ozone was still effective, which could be demonstrated by the reduction of colony count by half at these two positions, as shown in Fig. 17 and 18. However, the effectiveness of bacteria/fungi disinfection were reduced at position 3 on the floor next to a shoe shelf and position 5 on the floor below project screen.

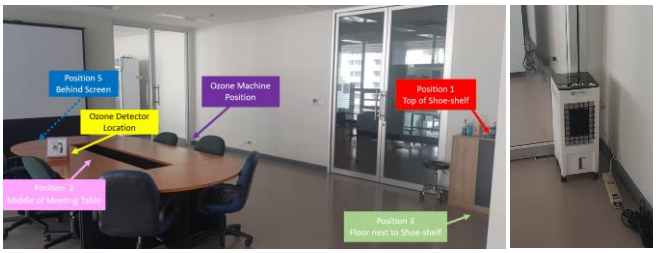

Fig. 16. Bacteria/fungi disinfection processes were performed in the meeting room at TUMIRL using the developed ozone machine.

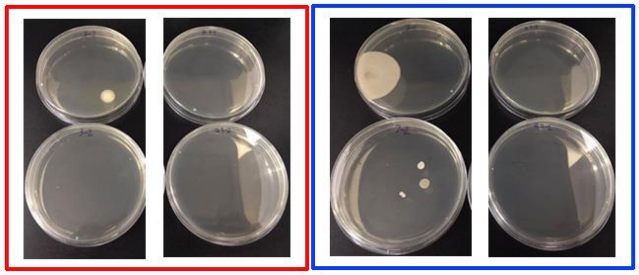

Fig. 17. *Position 1*: on top of the shoes shelf (Left red box) 2 duplicated control PCA plates with 1 colony in the left column and 2 duplicated PCA plates with no colony after ozone exposure in the right column. *Position 2*: in the middle of the meeting table (Right blue box) 2 duplicated control PCA plates with 1 and 3 colonies in the left column and 2 duplicated PCA plates with no colony after ozone exposure in the right column.

Before starting the disinfection process using ozone gas, there were 22-species of bacteria and 7-species of fungi, but after turning on the ozone machine for 50 minutes, only 11-species of bacteria and 2-species of fungi survived, which could be identified by the MALDI-TOF MS machine. The developed ozone machine could disinfect the following species of bacteria: 1) *Acinetobacter lwoffii*, 2) *Acinetobacter schindleri*, 3) *Acinetobacter ursingii*, 4) *Corynebacterium afermentans*, 5) *Corynebacterium amycolatum*, 6) *Dermabacter hominis*, 7) *Dermacoccus* spp., 8) *Kocuria rhizophila*, 9) *Massilia timonae* and *Massilia varians*, 10) *Micrococcus luteus*, and 11) *Staphylococcus warneri* as well as the following species of fungus: *Acremonium* spp. *Aspergillus* spp. and *Beauveria* spp. during these experimental tests.

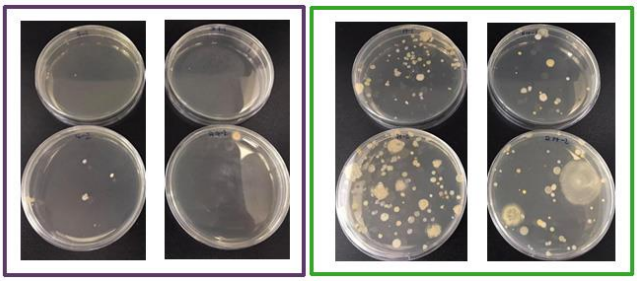

Fig. 18. *Position 3*: on the floor next to the shoes shelf (Left purple box) 2 duplicated control PCA plates with 1 and 5 colonies in the left column and 2 duplicated PCA plates with 1 colony in both plates after ozone exposure in the right column. *Position 4*: under the cleaning cloth on the coffee table (Right green box) control PCA plates with 59 and 113 colonies in left column and PCA plates with 22 and 47 colonies after ozone exposure in the right column.

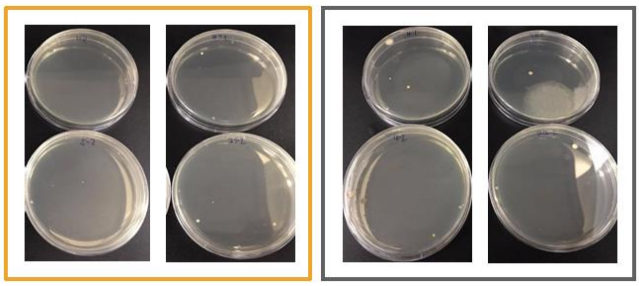

Fig. 19. *Position 5*: on the floor below projector screen (Left orange box) 2 duplicated control PCA plates with 1 and 2 colonies in the left column and 2 duplicated PCA plates with 1 and 4 colonies in the right column after ozone exposure. *Position 6*: on the high shelf (Right gray box) 2 duplicated control PCA plates with 4 and 2 colonies in the left column and 2 duplicated PCA plates with 3 and 1 colonies after ozone exposure in the right column.

5. Conclusion

The low-cost ozone machine was developed and experimentally tested for validating its virucidal efficacy according to the achievable total ozone dose as well as for evaluating its effectiveness on the bacteria/fungus disinfection from cultural tests using PCA plates after the ozonation process of 50-minute duration. The developed ozone machine consisted of 1) the DBD ozone-generator type that yielded sufficient Total Ozone Dose (TOD) for >90% viral inactivation within the 50-minute duration, 2) the timer, constructed from the microcontroller to control the relay for turning on-off the ozone generator with the LCD display, and 3) the centrifugal blower to thoroughly disperse ozone gas within the close and unoccupied room.

This developed ozone machine could experimentally attain the TOD within the range of 41.25-64.05 ppm-min, inside the non-ventilated 50 square-meter classroom, which was on the high-side of the >90% viral disinfection requirement. Amount of TOD was depended on the ambient temperature and relative humidity. Nevertheless, the achievable TOD within the 50-minute period inside the ventilated classroom still remained within the >90% viral disinfection limit. The waiting period of about 1.5 hours for safely re-entering the decontaminated room could be estimated from the exponential decay rate of OC until the value of OC was reduced below 0.1 ppm. Besides that the predicted Half-Life Times (HLTs) inside the non-ventilated classroom were experimentally estimated to be between 15 and 19 minutes, they were much lower than the ideal HLT that varied within a range of 650 and 715 minutes for the same ambient condition. For the ideal condition, the upper-limit ozone HLT inside the enclosed plexiglass cylinder were computed from Eq. (2). Several factors, contributed to the much smaller value of the experimentally predicted HLT, were indoor and outdoor air exchange through ceiling and door gaps as well as ozone absorption by different materials inside the room with various removable rates.

Finally, the effectiveness of gaseous ozone for disinfecting different bacteria and fungi, which were more resistant to ozone inactivation than most viruses, were validated using the cultural tests of PCA plates with and without ozone exposure during the 50-minute period. These PCA plates were placed at different radial distances and heights from the developed ozone machine inside the non-ventilated room. This ozone machine was very effective to inactivate both bacteria and fungi within the 5-meter radial distance in forward direction of the blower and within 2-meter height above this machine. Furthermore, clothes could absorb ozone gas very well so that bacteria/fungi could be substantially reduced during the ozone disinfection process.

5.1. Future Work

To further improve the efficacy of bacteria/fungi disinfection inside the non-ventilated room using this developed ozone machine within the 50-minute duration, at least two ozone machines should be placed at two adjacent corners such that flow of gaseous ozone can cross each other and disperse within the entire room area even more thoroughly. One of these machines should

direct ozone flow downward, while the other should direct flow of ozone gas upward.

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References

[1] United States Environmental Protection Agency. "Indoor Air and Coronavirus (COVID-19)." https://www.epa.gov/ coronavirus/indoor-air-and-coronavirus-covid-19 (accessed Jan. 10, 2022).

[2] M. Heßling, K. Hönes, P. Vatter, and C. Lingenfelder, "Ultraviolet irradiation doses for coronavirus inactivation—Review and analysis of coronavirus photoinactivation studies," *GMS Hyg. Infect Control*, vol. 15, pp. 1-8, May 2020.

[3] K. Dhama, S. K. Patel, R. Kumar, R. Masand, J. Rana, M.I. Yatoo, R. Tiwari, K. Sharun, R. K. Mohapatra, S. Natesan, M. Dhawan, T. Ahmad, T. B. Emran, Y. S. Malik, and H. Harapan, "The role of disinfectants and sanitizers during COVID-19 pandemic: Advantages and deleterious effects on humans and the environment," *Environ. Sci. Pollut. Res. Int.*, vol. 28, no. 26, pp. 34211-34228, Jul. 2021.

[4] L. Vallone and S. Stella, "Evaluation of antifungal effect of gaseous ozone in a meat processing plant," *Ital. J. Food Saf.*, vol. 3, no. 2, p. 1680, Jun. 2014, doi: 10.4081/ijfs.2014.1680. PMID: 27800339; PMCID: PMC5083869.

[5] Y. D. Porto, F. M. Trombete, O. Freitas-Silva, I. M. de Castro, G. M. Direito, and J. L. R. Ascheri, "Gaseous ozonation to reduce aflatoxins levels and microbial contamination in corn grits," *Microorganisms*, vol. 7, no. 8, p. 220, Jul. 2019.

[6] M. Huang, M. K. Hasan, K. Rathore, M. A. H. Baky, J. Lassalle, J. Kraus, M. Burnette, C. Campbell, K. Wang, H. Jemison, S. Pillai, M. Pharr, and D. Staack, "Plasma generated ozone and reactive oxygen species for point of use PPE decontamination system," *PLoS ONE*, vol. 17, no. 2, pp. 1-35, Feb. 2022.

[7] B. Fontes, A. Maria, C. Heimbecker, G. de S. Brito, S. F. Costa, I. M. van der Heijden, A. S. Levin, and S. Rasslan, "Effect of low-dose gaseous ozone on pathogenic bacteria," *BMC Infectious Diseases*, vol. 12, Dec. 2012, Art. no. 358.

[8] K. Rangel, F. O. Cabral, G. C. Lechuga, J. P. R. S. Carvalho, M. H. S. Villas-Bôas, V. Midlej, and S. G. De-Simone, "Detrimental effect of ozone on pathogenic bacteria," in *Microorganisms*, vol. 10, no.1, Dec. 2021, doi: 10.3390/microorganisms10010040.

[9] E. I. Epelle, A. Macfarlane, M. Cusack, A. Burns, B. Thissera, W. Mackay, M. E. Rateb, AND M. Yaseen, "Bacterial and fungal disinfection via ozonation in air," *Journal of Microbiological Methods*, vol. 194, pp. 1-10, Feb. 2022.

[10] N. Aich, S. Nama, A. Biswal, and T. Paul, "A review on recirculating aquaculture systems: Challenges and opportunities for sustainable aquaculture," *Innovative Farming*, vol. 5, no. 1, 2020.

[11] F. Ali, D. L. Lestari, and M. D. Putri, "State of the art: Ozone plasma technology for water purification," *Engineering Journal*, vol. 25, no. 1, pp. 177-186, Jan. 2021.

[12] A. A. Isikber and C. G. Athanassiou, "The use of ozone gas for the control of insects and micro-organisms in stored products," *Journal of Stored Products Research*, vol. 64, pp. 139-145, Oct. 2015.

[13] M. E. Dubuis, N. Dumont-Leblond, C. Laliberte, M. Veillette, N. Turgeon, J. Jean, and C. Duchaine, "Ozone efficacy for the control of airborne viruses: Bacteriophage and norovirus models," *PLoS ONE*, vol. 15, no.4, pp. 1-19, Apr. 2020.

[14] E. Grignani, A. Mansi, R. Cabella, P. Castellano, A. Tirabasso, R. Sisto, M. Spagnoli, G. Fabrizi, F. Frigerio, and G. Tranfo, "Safe and effective use of ozone as air and surface disinfectant in the conjuncture of Covid-19," *Gases*, vol. 1, pp. 19-32, 2021.

[15] K. K. K. LAM. "Ozone Disinfection of SARS-Contaminated Areas." https://www.oxidationtech.com/blog/wp-content/uploads/2020/04/Ozone-Disinfection-of-SARS-contaminated-areas.pdf (accessed May 25, 2021).

[16] J. B. Hudson, M. Sharma, and S. Vimalanathan, "Development of a practical method for using ozone gas as a virus decontaminating agent," *Ozone Sci. Eng.*, vol. 31, pp. 216-223, 2009.

[17] C. Tseng and C. Li, "Inactivation of surface viruses by gaseous ozone," *Journal of Environmental Health*, vol. 70, no. 10, pp. 56-63, Jun. 2008.

[18] W. J. Elford and J. van den Ende, "An investigation of the merits of ozone as an aerial disinfectant," *J. Hyg* (Lond), vol. 42, no. 3, pp. 240–265, May 1942.

[19] Centers for Disease Control and Prevention. "Ozone: Immediately Dangerous to Life or Health Concentrations (IDLH)." https://www.cdc.gov/niosh/idlh/10028156.html (accessed Jan. 10, 2022).

[20] United States Environmental Protection Agency. "Ozone Generators that are Sold as Air Cleaners." https://www.epa.gov/indoor-air-quality-iaq/ozone-generators-are-sold-air-cleaners (accessed Jan. 10, 2022).

[21] J. D. McClurkin and D. E. Maier, "Half-life time of ozone as a function of air conditions and movement," in *Proceeding of the 10th International Working Conference on Stored Product Protection*, 2010, pp. 381-385.

[22] C. J. Weschler, "Ozone in indoor environments: Concentration and chemistry," *Indoor Air*, vol. 10, pp. 269–288, 2000.
[23] M. Strejcek, T. Smrhova, P. Junkova, and O. Uhlik, "W Whole-Cell MALDI-TOF MS Versus 16S rRNA Gene Analysis for Identification and Dereplication of Recurrent Bacterial Isolates," in *Microbiol*, vol. 19, no. 9, p. 1294, Jun. 2018.
[24] L. Matthapan et al., "A novel technique of aluminum multiplier slide culture for fungal identification," *Sririraj Med. J.*, vol. 70, no. 5, pp. 438-441, 2018.
[25] G. Wen, Z. Liang, X. Xu, R. Cao, Q. Wan, G. Ji, W. Lin, J. Wang, J. Yang, and T. Huang, "Inactivation of fungal spores in water using ozone: Kinetics, influencing factors and mechanisms," *Water Res.*, vol. 185, p. 116218, Oct. 2020, https://doi.org/10.1016/j.watres.2020.116218.

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