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Investigating the Effectiveness of Ozone Based Air Purification System For Indoor Microbial Control

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Abstract:

Background: Global climatic change and the presence of particulate matter in the atmosphere has led to the greater need of maintenance of hygienic intramural habitats. Ozone has proved to be one of the possible approaches to address the disinfection of indoor habitats. Ozone, a powerful oxidizing agent, is an effective means of reducing microbial contamination in the ambient air. The current study aims at air disinfection with ozone technology that can be one of the crucial tools to maintain minimal microbial content in the atmosphere.

Materials and methods: A cost-effective indigenous ozone generator was designed and standardized to produce gaseous ozone in permissable concentration to decontaminate the ambient indoor space in a programmed 30 minutes cycle. This ozone technology was supplemented with centrifugal air flow system that helps to uniformly disperse the ozone gas across the standardized designated indoor space by utilizing dry ambient air as a precursor. Once the optimum ozone concentration is attained, the technology injects the ozone into the ambient indoor atmosphere which is to be disinfected. The microbial load sampling of pre-intervention ambient indoor air before the ozone treatment served as control. After the 30 minute ozone intervention the microbial load sampling was studied by the standard microbiological protocol to analyse the antimicrobial effect of ozone.

Results and discussion: The ozone gas observed to have a highly statistically significant disinfection ability ($p<0.0005$) as the ambient indoor air microbial sample showed decrease in CFU(Colony Forming Unit) count after ozonation.

Conclusion: The application of optimal ozone concentration to disinfect the habitat at the at-most safe means was materialized through ozone as an efficient disinfecting agent. The applications has vast implications as it can be installed in any of the day-to-day environments viz., hospitals, laboratories enclosed public spaces etc., where minimal CFU is expected and maintaining hygiene is a priority. Treatment with gaseous ozone proved beneficial with promising results by lowering the CFU of the microorganisms in the current study. The outcomes suggest that ozone disinfection is a potential substitute to conventional sanitizing methods with wider application when compared to the traditional chemical disinfectants.

Keywords: Ozone Concentration, Disinfection, Ambient indoor air sanitation, Microbial contamination

I. Introduction:

The newly discovered corona virus outbreak had a severe impact on human life, socially and economically. Viruses and Bacteria frequently cause health hazards in the society(1). The infection can spread through the airborne particles and droplets, finally come in contact with respiratory fluids, carrying the infectious agents and settling on various surfaces(2). Viable particles can spread diseases and remain airborne for a few hours in enclosed spaces(3). In terms of Immunization, effective disinfection such as environmentally safe and reliable methods are important. Ozone is a powerful and universal bactericidal/virucidal (4) disinfectant. The biochemical activity and infectivity of bacteria and viruses can become less virulent due to the presence of ozone in the atmosphere(1).

Ozone is essentially a newly formed and associated product of oxygen atoms. It is a highly hazardous gas for life since it is extremely reactive and has a high potential for oxidation(5) . Ozone has been used for many years to disinfect, and its powerful oxidizing capabilities are primarily responsible for its bactericidal effects, which have been well-documented(6). In essence, ozone is an association result of freshly generated oxygen atoms(7). Because of its intense reactivity and high propensity for oxidation, this gas poses a serious threat to life(8) controlled application towards the disinfection of viruses and bacteria can be explored where, an automated room disinfection systems could be used as an additional disinfection technique in hospital rooms(9) and settings to address this issue and reduce environment-borne transmission of infectious agents (10), where Ozone leaves no chemical residue and kills a wide range of bacteria, including *Listeria* and *E. Coli*, faster than conventional disinfectants(8). At the moment, methods such as ultraviolet (UV) radiation, ozone, hydrogen peroxide that has been aerosolized and vaporized are used for disinfecting rooms after the discharge of patients(10). In order to accomplish this, an ozone-based air purification device is provided by an embodiment of the present invention(11). The objective of the present invention is to oxidise and remove contaminants present in the contaminated air(8). Furthermore, the FDA certified it as GRAS (Generally Regarded As Safe) in 1997, that guarantees its application as a disinfectant (5). As per the survey mentioned above, it is clear that safe and effective methods are needed in order to ensure that air environments, room surfaces, and sanitation materials are disinfected in case of an unforeseen incident or pandemics(12).

Ozone's mechanism of disinfection:

The combination of chemical, physical, and biological factors make up the ozone disinfection mechanism more effective and efficient(1). In addition to the splitting of the third oxygen atom, an ozone molecule has a high oxidation strength(13). This characteristic renders it highly efficacious in eliminating microorganisms(14). Ozone has the potential to be effective against viruses, fungi, bacteria, protozoa, and vegetative forms of bacteria(5). Ozone disrupts the proteins and lipids in the viral envelope and spike synapses, impairing viral integrity and rendering the virus inactive(13). Through a chemical reaction with the proteins in the bacterial cell wall, ozone easily penetrates the wall, oxidizing intracellular enzymes, RNA, or DNA to kill the pathogens(1). In aqueous systems, ozone reacts with different chemical compounds in two distinct but coexisting ways: directly by molecular ozone reactions in one mode, and another through free radical-mediated destruction(15). The effectiveness of ozone gas disinfection is dependent upon multiple factors, that includes ozone concentration, contact duration, temperature, and relative humidity of the ambient air(3).

Ozone sterilization machine:

The main objective for this low cost ozone generating machine is to effectively remove bacteria or viruses from the environment. Further, the ozone machine's design and construction were intended to use gaseous ozone to sterilize unoccupied and contaminated classrooms or meeting rooms up to 100sft. A centrifugal blower with a high flow rate could produce uniform ozone gas dispersion, allowing low doses of ozone to be applied to large surfaces in the enclosed space.

II. Materials and Methods:

In order to find out if ozone can effectively decrease airborne microorganisms, an approximately 100 m2 conference room was used for the experiment. The process of disinfection is carried out in the absence of humans as bacteria and viruses are the targeted agents to kill by a high concentration of ozone. The ozone generator in the room can generate 5g of ozone as 1g/hr. The designed ozone generator's capacity can sustain a high ozone concentration of 0.5–5 ppm inside. To ensure the distribution of ozone throughout the space, a fan or air conditioner was placed.

The following is the technical procedure that was followed to ozonate the room: The Tryptone Soy Agar (TSA) plates were prepared aseptically. Before performing disinfection process, 5 plates of open-lid sterilized Tryptone Soy Agar (TSA) were placed at the multiple locations with various distance and exposed for different time intervals from the developed ozone machine at a room temperature for 35 minutes which these sets were used as controlled reference for each location . After the room was exposed to ozone gas for 35 minutes with ventilation by keeping on the air conditioner or fan for the circulation of ozone in the room and processed in the same way as the control references, the ozone generator will then be switched off. The ozone in the room starts to thermally decompose on its own when the concentration become less than 1 ppm. Following that, Petri dishes were placed inside for ten minutes. The distance between the Petri dishes is approximately 4 foot distance from the ozone generator. Petri dishes were kept in an ozone-treated room for ten minutes. The petri dishes were then incubated at 37°C for 24 hours. Subsequently, the petri dishes were examined visually to determine the presence of number of colonies.

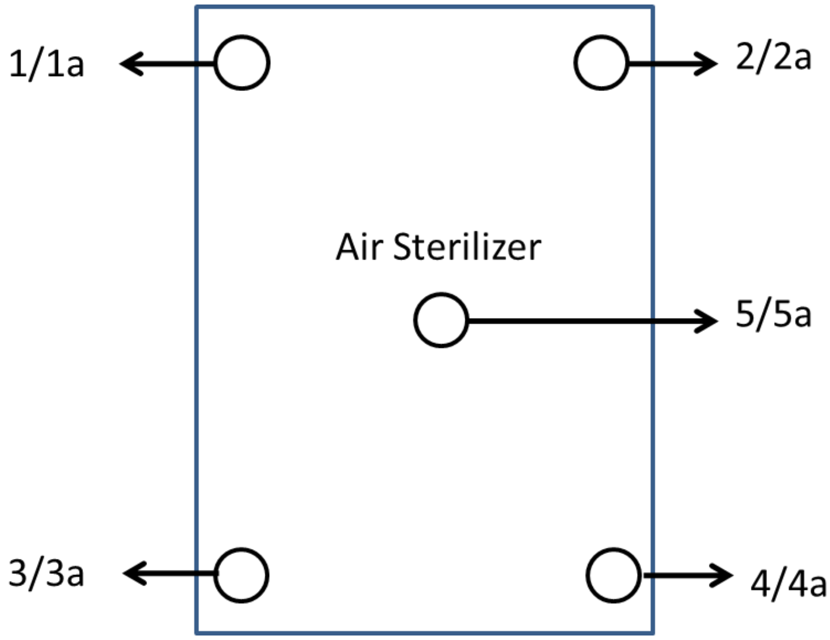

Fig 1: Rough diagram of petriplates located in the conference room

III. Results:

The total amount of airborne bacteria in the conference room was measured before and after each ozonation. Table 1 lists the disinfection efficiency of ozonation at various concentrations.

Table 1. Reduction of Airborne Bacteria after Ozonation based on ozone concentration

Ozone concentration	**0.5ppm**	**2.5ppm**	**5ppm**
Before Ozonation	397	445	412
After ozonation	136	42	37
Reduction (p value)	<0.0001	<0.0001	<0.0001

p<0.0001 based on paired t-test using using GraphPad Prism Software v.5,0. There was statistically significant reduction in airborne bacteria after Ozonation at all the concentration viz. 0.5ppm (p<0.0001), 2.5ppm (p<0.0001) and 5 ppm (p<0.0001)

Table 2: Reduction of Airborne bacteria after ozonation based on the distance of Petri dishes from Ozonator

Petri dishes exposure time for ozone	**2 minutes**	**5 minutes**	**10 minutes**
Before ozonation	186	239	435
After ozonation	28	41	38
Reduction (p value)	<0.0001	<0.0001	<0.0001

p<0.0001 based on paired t-test using using GraphPad Prism Software v.5,0. There was statistically significant reduction in airborne bacteria after Ozonation at all the exposure time of Petridishes viz.2mins (p<0.0001), 5 mins(p<0.0001) and 10 mins (p<0.0001).

In contrast to the controlled TSA plates without exposure to gaseous ozone, the results of the bacteria and fungal culture tests showed that, after a 35-minute ozone exposure, ozone concentrations between 3 and 4 ppm could successfully eradicate bacteria in the non-ventilated meeting room.

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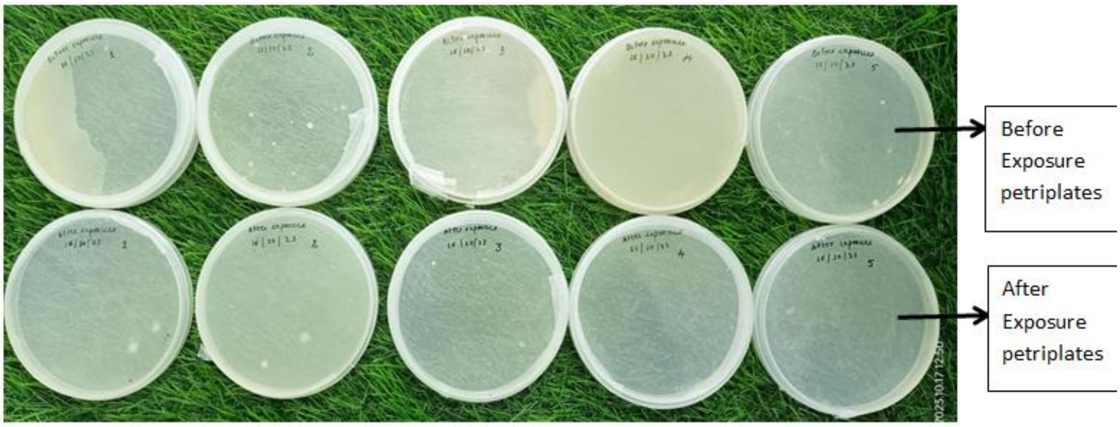

Fig 2: Comparison in reduction of bacteria in Tryptone Soy agar petri dishes of before and after ozonation

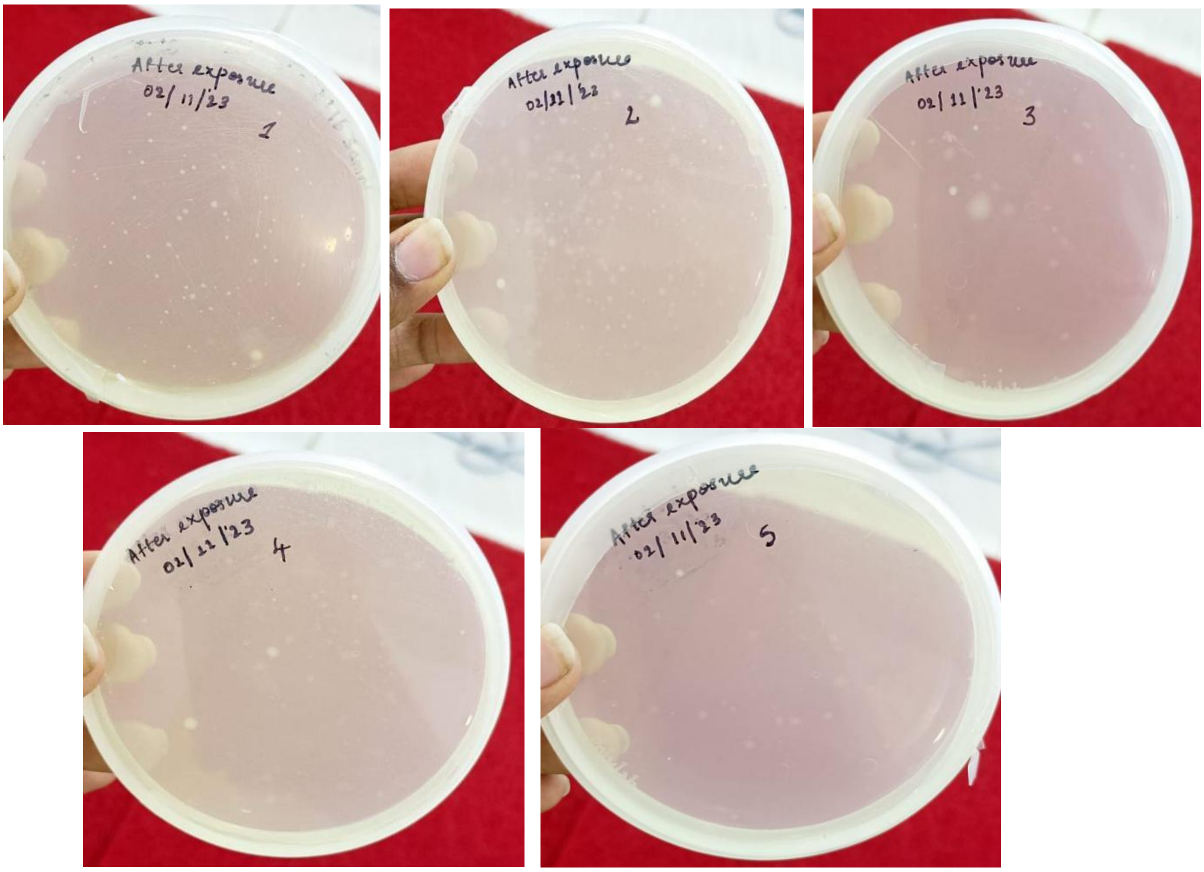

Fig 3: Reduction of bacteria in Tryptone Soy Agar Plates after ozonation for 35 minutes.

Ozone gas can effectively inactivate bacteria or fungi at positions 3, 4 and 5 of TSA plates because they are placed in front of the blower and within a 5-meter radial distance. The colony count at positions 1 and 2 was reduced by half, indicating that the gaseous ozone was still effective in inactivating bacteria and fungi, despite their location in the opposite corner of the room from the ozone machine.

IV. Discussion:

The study's findings indicate that the ozone-based air purification system has a notable impact on reducing airborne bacteria in an enclosed environment. The introduction emphasized the urgency of developing effective disinfection techniques in response to health hazards such as viral outbreaks. Ozone, known for its powerful bactericidal and virucidal properties, was explored as a potential solution.

The results presented in Table.1 showcase a substantial reduction in airborne bacteria after ozonation at various concentrations. Higher ozone concentrations, such as 5 ppm, demonstrated a more significant reduction compared to lower concentrations, supporting the effectiveness of ozone in disinfection. The effectiveness of ozone and relative humidity combinations for the inactivation of various airborne microorganisms was tested by Dubuis ME et. al(16).

Table 2 further details the reduction in airborne bacteria concerning the distance of Petri dishes from the Ozonator and the exposure time. The results indicate that ozone exposure time significantly influences the reduction of airborne bacteria, reinforcing the importance of proper application and duration for optimal disinfection. The findings are consistent with those published by Zucker et.al in 2021(17), who conducted their research in a reaction chamber furnished with a small table and cabinet in order to induce indoor contamination.

The successful eradication of bacteria in the non-ventilated meeting room after a 35-minute ozone exposure is a promising outcome. The ability of ozone to effectively inactivate bacteria, at various positions within the room, even at a distance from the ozone machine, demonstrates the widespread reach of its disinfection capabilities. The reduction in colony count at positions 3 and 4, despite being in the opposite corner of the room from the ozone machine, highlights the mobility and efficacy of gaseous ozone in disinfection.

The majority of studies that have been published in the literature (18) and (19) have focused on the analysis of laboratory-simulated environmental conditions in small chambers or cabinets that have experimental conditions set to room temperature and relative humidity greater than 55%. According to Yao et al. 2020, (20) SARS-CoV-2 was rendered inactive through gaseous ozone treatment at room temperature (25C) and relative humidity levels between 60 and 80%. The ozone treatment was conducted at concentrations of 1.0 ppm for 60 minutes and 6.0 ppm for 55 minutes. It's essential to acknowledge the limitations of the study, including the controlled experimental conditions and the need for further research to validate these findings in real-world settings. Additionally, factors like ozone concentration, exposure time, and room characteristics play crucial roles in the effectiveness of ozone-based disinfection, emphasizing the importance of tailored application methods.

The study also provides valuable insights into the efficacy of ozone-based air purification systems in reducing airborne bacteria. The results support the potential use of ozone as an environmentally safe and efficient disinfection technique, particularly in enclosed spaces where traditional methods may fall short. Further research and practical implementations are warranted to establish the broader applicability and safety of ozone-based disinfection in diverse settings. These TSA plates were positioned inside the non-ventilated room at various heights and radial distance from the developed ozone machine. Within a 2-meter height range above the machine and a 5-meter radial distance in front of the blower, ozone machine proved to be highly effective in rendering both bacteria and fungi inactive.

V. Conclusion:

Ozone is a green product that protects the environment with a wide range of highly efficient disinfectants. The use of a small-sized ozone disinfection in home environments can effectively prevent and control skin diseases as well as Influenza. The practical significance of ozone disinfection lies in its ability to prevent and manage epidemic diseases. The potent role that ozone sterilizers play in healthcare and epidemic prevention should be focused more in future for the clinical research on infectious diseases.

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