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Bactericidal and Fungicidal Efficacy of Chlorine Dioxide in Various Workspaces

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Abstract

Previously, we demonstrated the virucidal efficacy of low concentration chlorine dioxide (ClO_2) gas in room settings. The purpose of these studies was to evaluate novel ClO_2 formats as potential biocidal interventions for real world congregate settings and air systems. Three types of studies were conducted to determine the efficacy of ClO_2 in reducing bacteria and mold in various workspaces: hard and soft surfaces (gymnasium & equipment), aerosol (in-room), and within a laboratory environment. The study demonstrated that ClO_2 was highly effective against both bacteria and mold with reduction ranging from 85.0% - > 99.4% for bacteria and >99.4% for yeast and mold. Treatments on hard and soft surfaces (gymnasiums and sports equipment), reduced bacteria by an average of 90% - 95%. The following treatments were applied overnight: 1) hard surface spraying with dilute ClO_2 solutions, 2) carpet and tumbling treatments with powdered ClO_2 releasing impregnates, and 3) HVAC treatment and overall room deodorization with low dose ClO_2 gas from controlled releasing sachets. The in-room study treating air with a ClO_2 filtration media also indicated significant air and surface room efficacy, with an average of 94% reduction in bacteria after 24-hour, and 99.4% reduction in mold after 24-hours. In a related air study, a biological combination of *Raoultella terrigena* and *Staphylococcus aureus* was injected as a bio-aerosol into a 4-inch diameter pipe with air flowing at approximately 1200 ft/min. Dry ClO_2 gas was introduced into the air flow to achieve an effective concentration of 5 or 10 ppmv. Air samples were collected at sampling ports downstream from the fan at 10, 22, 55 and 100 ft along the pipe and used to evaluate changes in airborne bacteria and mold. Testing was conducted in a laboratory setting at ambient conditions. The data showed ClO_2 gas reduced viable organisms at both gas concentrations, and indicated that reductions were higher for 10 ppmv concentration, and longer pipe runs. In a final study, laboratory appli-

cation of gaseous chlorine dioxide was tested. Low gas release filter testing demonstrated significant surface reductions of airborne bacteria with an overall average 99.4% reduction in the 24-hour testing period. Higher gas treatments of a class II biological cabinet reduced bacillus spores on steel coupons throughout cabinet by 6 log. ClO_2 was effective as a bactericidal and fungicidal treatment providing significant reduction in both surface and air. Novel product delivery forms may be useful for rapidly disinfecting air and solid surfaces in complex congregate settings.

Keywords

Chlorine Dioxide, Bactericidal Activity, Fungicidal Activity

1. Introduction

Consistent inhalation of bacteria and mold has been associated with a myriad of respiratory conditions ranging from asthma and rhinosinusitis to bronchitis and lung infections [1]. This is particularly precarious for workers who spend extended periods of time in contaminated spaces inhaling respiratory contaminants. Office buildings, schools, and many other nonindustrial buildings may develop moisture and dampness which often leads to the growth of mold, fungi, and bacteria [1]. Common sources of moisture in buildings include plumbing, roofs, window leaks, flooding, condensation on cold surfaces, poorly maintained drain pans, and wet foundations [2]. The term "mold" encompasses groups of fungi common on wet materials, and yeast, which were observed in this study [1]. Long-term exposure to molds and their metabolic by-products may be associated with a variety of adverse health consequences, making workspaces a particularly important setting to contain mold [1]. In addition to mold, bacterial spores are prone to develop in almost any workplace setting, especially in shared spaces and on equipment. Certain bacteria can remain on surfaces for longer than a week, and exposures can lead to many illnesses and infections. A 2011 study collected 400 samples in various public settings (offices, homes, supermarkets, etc.) and reported robust bacterial colonies on common everyday surfaces [5]. The article noted the necessity for a gentle cleaning solution that would not damage surfaces, keyboards, or computers [3].

1.1. Background and Uses of Chlorine Dioxide

Discovered in 1814, chlorine dioxide (ClO_2) is a yellowish-brown gas at standard temperature and pressure. Below 52˚F, ClO_2 is a liquid, whereas above 52˚F it turns into a gas [4]. As a disinfectant, it is mainly used in liquid form. Standard industrial uses of ClO_2 include bleaching cellulose, paper-pulp, flour, leather, fats and oils, textiles, and beeswax [4]. In 2001, it was used to decontaminate numerous public buildings following the release of anthrax spores in the United States [4]. It is also used in water purification, odor and taste control for potable

water, cleaning and de-tanning leather, among many other uses [4]. Aqueous chlorine dioxide was found to be safe when ingested in low doses, but when ingested in high doses, it can cause adverse hematologic and renal effects [4].

ClO_2 is a strong oxidizing agent that rapidly destroys viruses and is used for bleaching, potable water disinfection, killing microbes on food, and sterilizing medical equipment [5] [6] [7]. The Environmental Protection Agency [EPA] first registered the aqueous form of ClO_2 for use as a disinfectant and a sanitizer in 1967, and as a sterilant in 1988 [8]. In May 2020, the EPA listed ClO_2 as an approved hard surface disinfectant for use against SARS-CoV-2 [9].

ClO_2 is also approved for use in the agricultural industry as a sanitizing rinse compound for produce and for cleaning food processing equipment. Notably low-level ClO_2 gas was approved by the EPA and FDA for reducing spoilage and pathogens on produce surfaces. In clinical settings, ClO_2 gas is used for sterilization of medical devices and disinfection of patient hospital rooms. There are related and extended uses of low-level ClO_2 gas in commercial environments for odor control and for reducing odor causing bacteria.

1.2. Safety of Chlorine Dioxide

The safety and efficacy of ClO_2 disinfectant products have been approved by the United States Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) for a variety of antimicrobial uses. The Occupational Safety and Health Administration (OSHA) and EPA have both set the allowable time-weighted average exposure of ClO_2 at 0.1 ppm over an 8-hour work shift, with OSHA increasing the concentration to 0.3 ppm for short-term 15-minute work periods. Due to the concentration standards set by the EPA, FDA and OSHA, the average worker is not likely to encounter ClO_2 at dangerous levels using approved products or site SOPs.

Numerous studies have found ClO_2 to be safe in low concentrations, such as a 6-month study involving 0.05 ppm (or 0.1 ppm) exposure to rats for 24 hours/day 7 days/week, in which no ClO_2-related toxicity was observed after a battery of toxicological examinations [10]. In another observation, a solution containing 50 ppm ClO_2 was investigated in a rabbit ocular irritation test [11]. The cornea, iris, and conjunctivae were evaluated and concluded that single ocular applications with 0.1 mL of 50 ppm of ClO_2 solution did not cause ocular irritation in rabbits [11]. In the same study, an inhalation toxicity test was observed using 0, 10, and 20 ppm which was administered as mist via humidifier in an airtight box containing five mice [11]. The test indicated no abnormality and no mortality for the control and test components withing 24 hours [11].

ClO_2 is typically not shipped in pure form and therefore the average individual is not likely to have access to concentrated products. New commercially available products have emerged that can accurately release low levels of ClO_2 gas in workplace settings, and as a result dramatically mitigate unwanted exposures to dangerous levels of ClO_2.

1.3. Chlorine Dioxide for Hard/Soft Surfaces

Evaluating the bactericidal efficacy of novel low release ClO_2 products for treating air, and/or hard and soft surfaces could provide significant benefits in congregate settings where bacterial growth is rich, such as fitness facilities, classrooms, nursing homes, fire stations, jails etc. Bacteria contamination on shared athletic gear or in athletic facilities has become a concern for high school, college, and professional athletic programs. Certain types of athletic gear are notoriously hard to clean without damaging the equipment because it is often made from porous material (e.g. leather, plastic foam) or is irregular in shape (e.g. hockey protective pads, football helmets, lacrosse gloves). Aerosol cleaners and liquid disinfectants often cannot penetrate the fabric surface covers to treat bacterial colonies and overuse may degrade the material reducing its primary function as personal protective equipment (PPE) for sports.

1.4. Aerosols

The transmission of infectious microbes via bioaerosol may be endemic and cause sporadic infections and outbreaks, resulting in significant concern for human health [12]. Low levels of ClO_2 gas may be useful in treating bioaerosols because of its broad-spectrum efficacy and high solubility in water [13]. ClO_2 possesses strong oxidizing activity, enabling the inactivation of bacteria, fungi, and viruses [13]. ClO_2 gas released from its aqueous solution has been used as a sanitizer and deodorant for room air [13].

A 2009 study observed lower rates of student absenteeism due to general illness (such as respiratory viral diseases) in a Japanese school by placing three commercial tabletop ClO_2 deodorant canisters in each classroom [12]. After 38 consecutive school days the rate of absenteeism appeared lower in classrooms where the ClO_2 devices were placed (1.5%) compared to the classrooms where such devices were not placed (4.0%) [13]. Based on this observation, it was determined that ClO_2 gas, at a concentration low enough not to harm humans, lowers the incidence of respiratory infections by inactivating airborne microorganisms within an enclosed space [13].

1.5. Sporicidal Efficacy of Chlorine Dioxide in a Controlled Laboratory Environment

Chlorine dioxide is a highly water-soluble gas that quickly partitions to moisture rich areas (*i.e.* bioaerosols and damp surfaces) which are known to promote survival and growth of microorganisms [12]. This quality also makes ClO_2 very efficient at eliminating mold spores. One study evaluated various fungal species in a controlled environment to understand the effects of chlorine dioxide on fungal growth when exposed to levels of 0 ppm, 500 ppm and 1000 ppm of ClO_2 gas [14]. In the levels of 500 ppm and 1000 ppm, *P. chrysogenum*, *S. chartarum*, and *C. cladosporioides* were unable to be cultured, indicating that the treatments were successful in completely inhibiting the culturability of these organisms [14].

2. Materials/Methods

2.1. Hard & Soft Surface Experiments

Two studies were conducted to observe the effects of various low releasing ClO_2 on hard and soft surfaces. In the first study, ClO_2 was used to reduce bacteria and odor on hard/soft surfaces throughout a gymnastics facility. The experiment was performed overnight, and the following treatments were applied: 1) hard surface spraying with dilute ClO_2 solution (ICA-TriNova LLC, Cleaner Sachets); 2) carpet and tumbling treatments with powdered impregnates (ICA-TriNova LLC ZeoSorb) releasing ClO_2; 3) HVAC treatment and overall room deodorization with low concentration ClO_2 gas releasing sachets (ICA-TriNova LLC Maintenace). Pre and post treatment surface swabs were taken to verify the bacteriocidal effects on various surfaces.

The second study took place in a high school locker room, where seventy-sets of helmets and shoulder pads were sealed in individual heavy-duty garbage bags with low level gas releasing sachets, (ICA-TriNova LLC Wipeout), and for 12 hours. The sachets were designed to release 100 mg of ClO_2 on the equipment. The study was undertaken to examine the effect of ClO_2 treatment on odor and bacteria reduction on the athletic gear. Six (6) sets of gear were selected randomly for microbiological analysis. Soft surfaces of shoulder pads were swabbed 8 - 12 times with a culture swab before and after treatment. Swabs were rubbed on sterile Trypticase Soy Agar (TSA) plates and placed in a 37˚C incubator and observed for growth after 48 hours.

2.2. Bioaerosol Experiment

Cultures of *Raoultella terrigena* (Rt) and *Staphylococcus aureus* (Sa) were grown in a tryptic soy broth for 18 - 24 hours at 36.5˚C and centrifuged at 4400 rpm for 10 minutes, then suspended in phosphate buffered saline. The biological cocktail of Rt and Sa was injected as a bioaerosol into a 4”-diameter pipe with air flowing at an approximate velocity of 1200 ft/min. A fan connected to one end of the 4” PVC pipe was used to create a flowing system. ClO_2 was produced using impregnates, (ICA-TriNova, LLC Wipeout), mixed in a breathable sachet. Dry ClO_2 gas was introduced into the air flow to achieve an effective concentration of 5 or 10 ppmv. Air samples were collected at sampling ports downstream from the fan at 10, 22, 55 and 100 ft along the pipe and used to evaluate changes in airborne bacteria and mold. A control study without ClO_2 gas injection was repeated two times to create a baseline of data without treatment. During the treatment tests, the container with the powders was rotated at a 45-degree angle to help introduce gas into the blower intake as the powders were agitated and reacted.

2.3. Laboratory Experiments

Two experiments were conducted in laboratory settings. In the first experiment, air was treated with a low release ClO_2 filtration media (ICA-TriNova LLC, Stay-

fresh). The StayFresh container, was placed at the center of a laboratory space of approximately 1300 cubic feet. The treatment and control laboratory rooms were held at ambient conditions. The room was used for normal work activities with people moving periodically in and out. A simple fan was used to push air through the oxidizing media and into the operating laboratory space to create an effective gas concentration of 0.1 ppmv by discharging approximately 1 mg/min release from the container. Three inoculated *E. coli* slides were placed randomly in the treatment room and collected after four-hour exposures. Air samples were taken at 4 hours and 24 hours of operation. Air samples were taken in both treated and control rooms.

In the second laboratory study, a Class II biological cabinet was used to observe the sporicidal efficacy of ClO_2. To isolate the internal cabinet surfaces the upper filter vent was sealed, and vinyl sheeting was used to tightly cover the front of the cabinet during the fumigation. The cabinet's chamber was opened to access the HEPA filters. Six ampules containing 1,000,000 CFU (Colony Forming Units) of bacillus atrophaeus spores were placed in the cabinet at the following locations: underneath main chamber grate on left hand side, underneath main chamber grate on right hand side, atop HEPA filter on left hand side, and atop HEPA filter on right hand side. Control vials were placed outside and on each sides of the cabinet. Vials change color based on their reaction, with yellow indicating a positive reaction, and green indicating no reaction. The cabinet's chamber was pre-humidified for 5 minutes using a sterile water vaporizer, increasing the humidity from 31.2% to 46.0%. ClO_2 release media were combined in an open cup (ICA-TriNova LLC Wipeout) placed in the cabinet and mixed. The cabinet's blower was activated and allowed to circulate internal air for 15 minutes, then deactivated along with the humidification system and left undisturbed for 2 hours and 25 minutes. The theoretical peak ClO_2 concentration in the cabinet at that time was 1 mg/L. The cabinet was then vented, and the test and control ampules were retrieved and placed in a 36.5˚C incubator for 48-hour.

3. Results and Discussion

Chlorine dioxide was effective at eliminating bacteria in all three experiment types. On hard/soft surface treatments, ClO_2 produced an average of ~93% reduction in bacteria across seven types of surfaces in the overnight gymnasium experiment (**Figure 1**). In the second surface study, treatment samples showed over ~99% of bacteria eliminated from athletic gear; in some instances, (e.g., Set #4, #6), very little bacteria were recovered from the equipment (**Figure 2**). **Figure 2** illustrates the percent reduction in bacterial colony counts on six sets of shoulder pads and helmets. The gear also had a noticeable reduction in odor which was noted as resembling that of a "fresh" odor post-treatment.

The results of the bioaerosol experiment indicated that ClO_2 was effective in treating *Staphylococcus aureus* (Sa) and *Raoultella terrigena* (Rt) (**Figure 3**). The

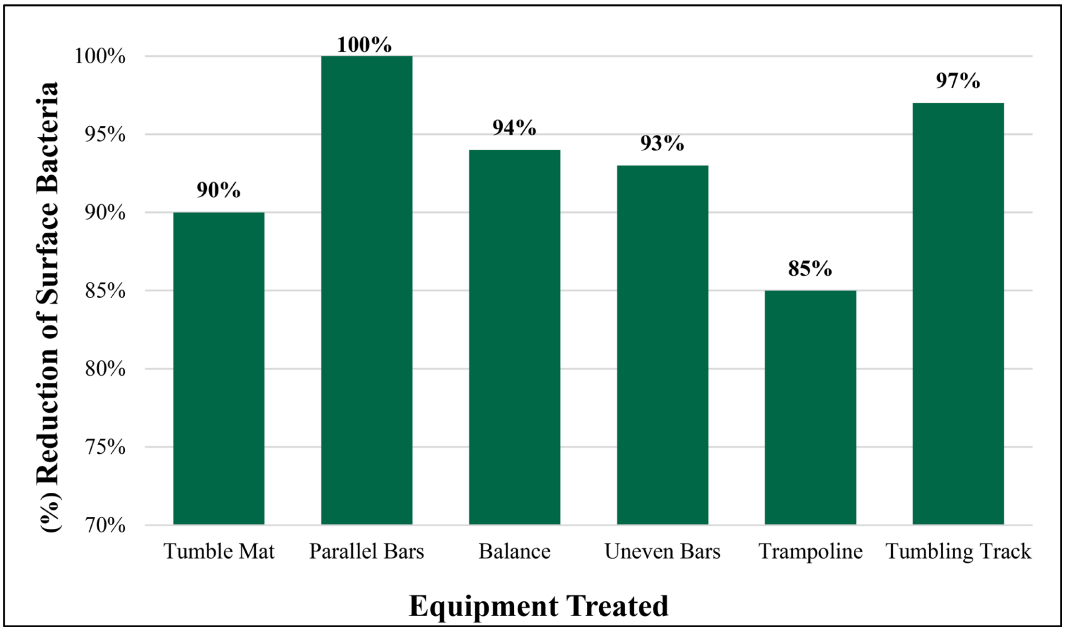

Figure 1. Gymnastic facility overnight observation.

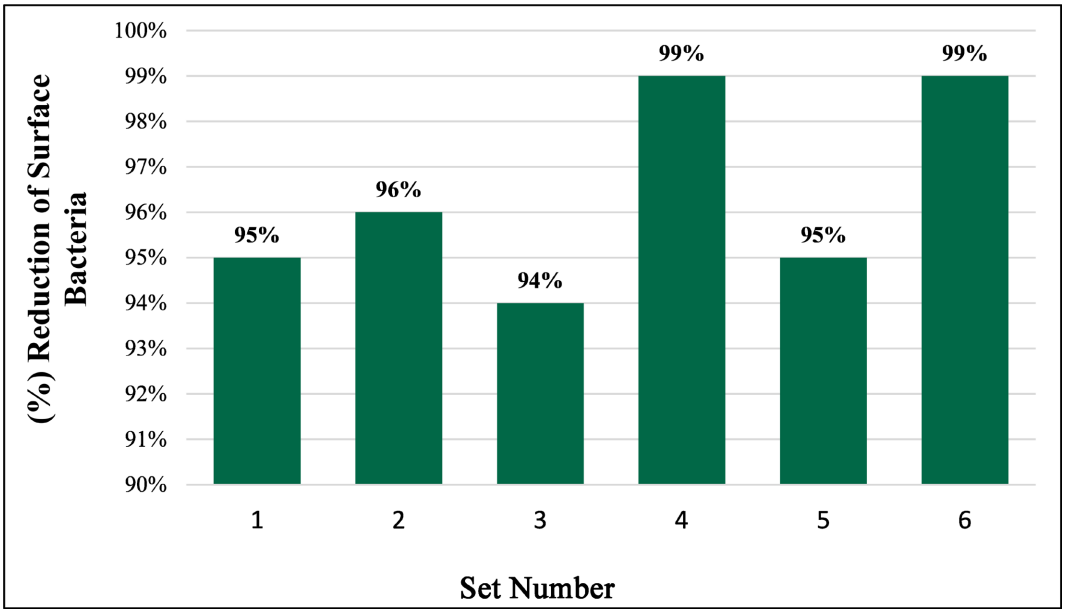

Figure 2. Shoulder pad and helmet overnight observation.

data showed ClO_2 gas increasingly reduced viable organisms along the length of the pipe run. Rt reductions were approximately 80%, and Sa 60% for these conditions. The shorter residence times resulted in lower reductions with higher variability likely due to inefficient mixing at the point of contact. This data shows how effective low-concentration dry chlorine dioxide gas can be at reducing microorganism contaminated bioaerosols in flowing air with very short contact times.

Figure 4 illustrates the results of the laboratory experiment, in which there were significant contaminant reductions on plate surfaces exposed to low level

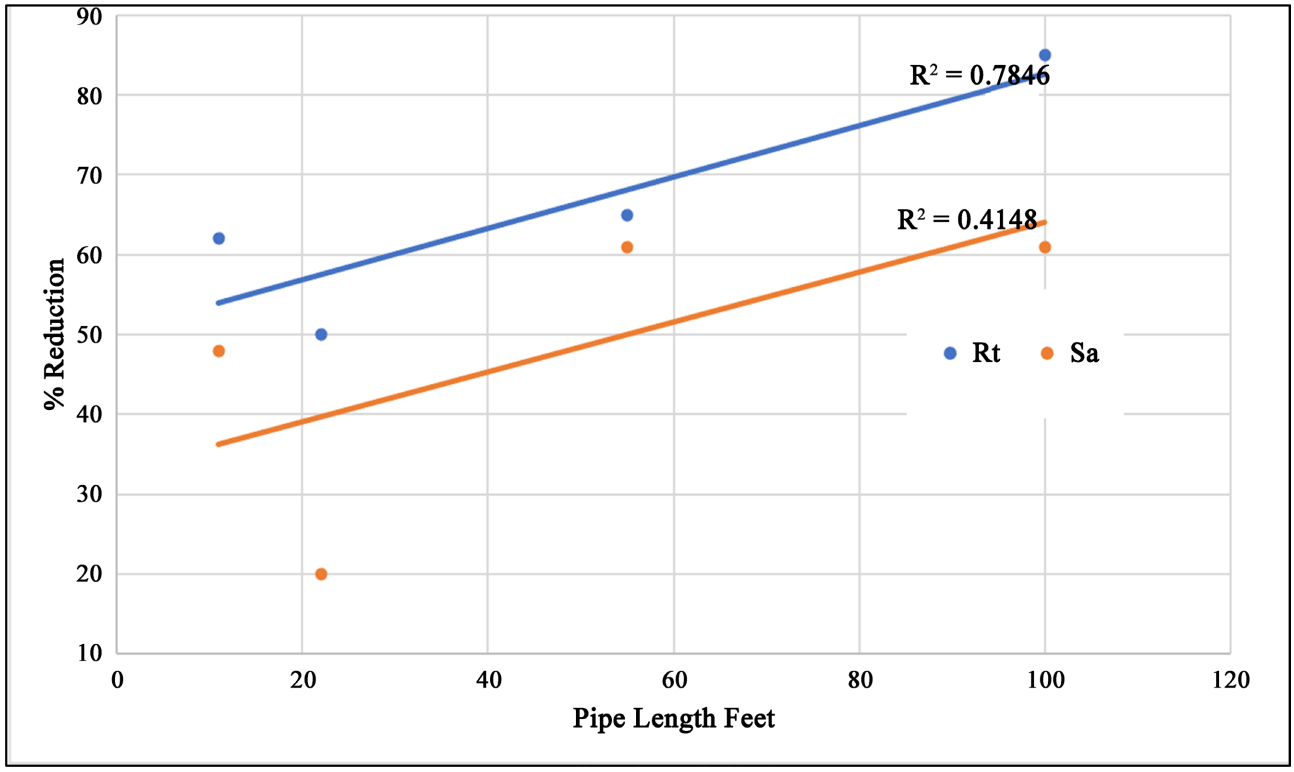

Figure 3. Bioaerosol in flowing air with low level dry ClO_2.

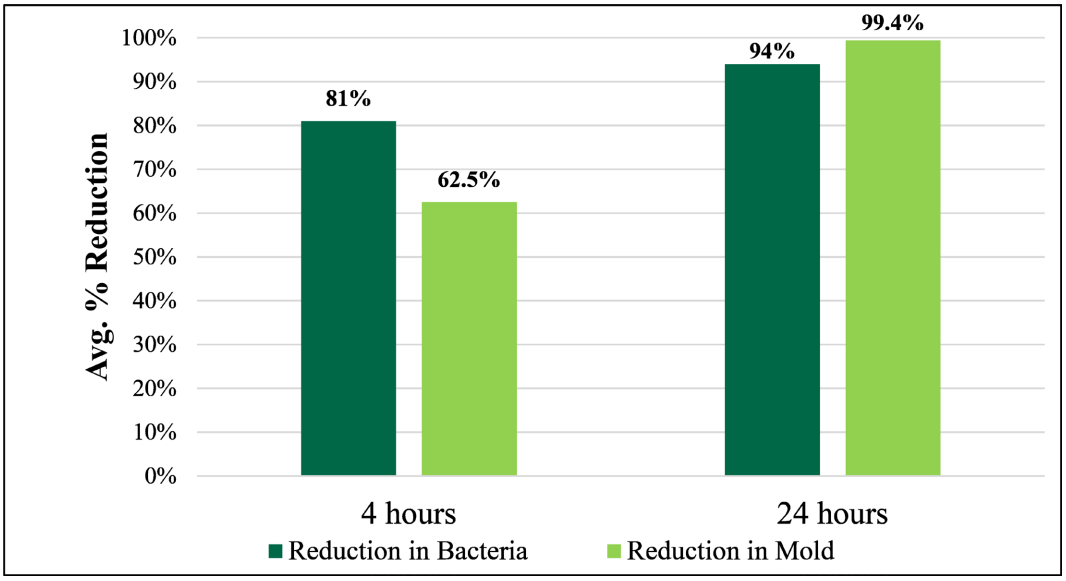

Figure 4. Low Level ClO_2 treatment of laboratory room air.

ClO_2 emitted from an impregnated filter system.

Exposure to low levels (1 ppmv) of ClO_2 reduced natural air bacteria and molds in the laboratory by greater than 60% and 90% in 4- and 24-hour observations respectively (**Figure 4**). This result reflects the room being open to the flow of typical work and people. This data also suggests bacteria showed greater reduction in shorter contact times than did mold spores. Longer operating times

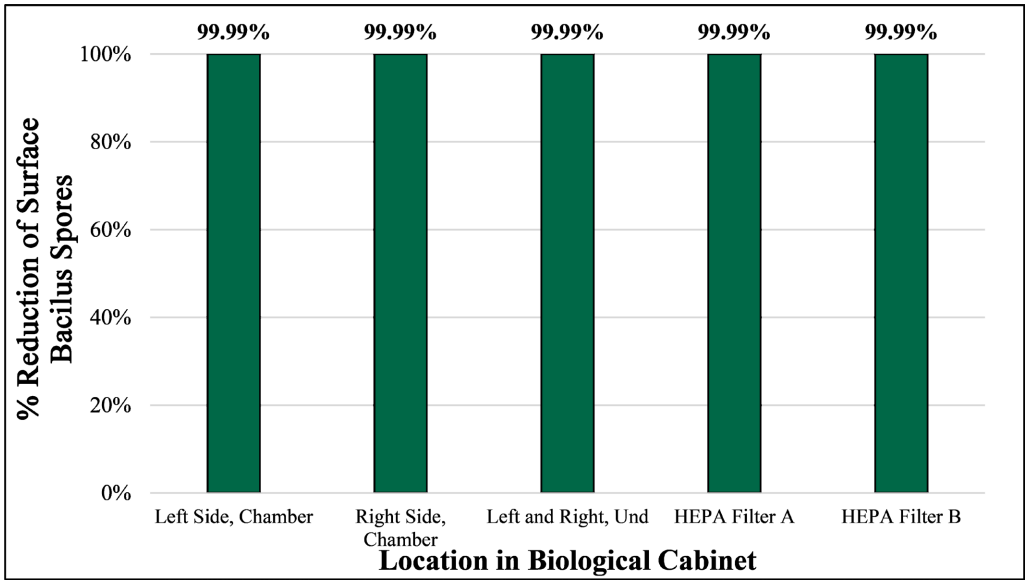

Figure 5. High Level Dry ClO_2 Gas in Class II biological cabinet.

showed that more significant reductions of both molds and bacteria could be achieved.

The Biological Cabinet study results also indicated significant efficacy albeit at a much higher ClO_2 gas concentration. Greater than 6 orders of magnitude reduction of ampules containing *B. atrophaeus* spores were observed at all sampling sites (**Figure 5**). All vials placed in the cabinet and treated with gas displayed no color change (indicating no reaction), including the spore sampling tubes placed on top of the HEPA filter system. Notably, the latter indicates free flow of gas through the filter and most likely an equivalent biological reduction inside and on filter fibers. This is an important finding as HEPA filters are known to support microbiome formation after extended periods of use [15].

4. Conclusion

Chlorine dioxide may be an effective bactericidal and fungicidal treatment across various workspaces based on these observations. These experiments allow for greater understanding of this treatment across many different surfaces, and in various settings. This study also introduces unique product formats of chemical delivery that increase the utility and safety of applying chlorine dioxide gas. Understanding the treatments in which ClO_2 is most effective will facilitate the development of opportunities to use this disinfectant in areas with high levels of viral, bacterial, and fungal contamination. These observations are valuable in understanding and developing new methods to utilize ClO_2 as a disinfectant in various workplace settings.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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