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Potential Biodefense Model Applications for Portable Chlorine Dioxide Gas Production

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Development of decontamination methods and strategies to address potential infectious disease outbreaks and bioterrorism events are pertinent to this nation's biodefense strategies and general biosecurity. Chlorine dioxide (ClO_2) gas has a history of use as a decontamination agent in response to an act of bioterrorism. However, the more widespread use of ClO_2 gas to meet current and unforeseen decontamination needs has been hampered because the gas is too unstable for shipment and must be prepared at the application site. Newer technology allows for easy, onsite gas generation without the need for dedicated equipment, electricity, water, or personnel with advanced training. In a laboratory model system, 2 unique applications (personal protective equipment [PPE] and animal skin) were investigated in the context of potential development of decontamination protocols. Such protocols could serve to reduce human exposure to bacteria in a decontamination response effort. Chlorine dioxide gas was capable of reducing (2-7 logs of vegetative and spore-forming bacteria), and in some instances eliminating, culturable bacteria from difficult to clean areas on PPE facepieces. The gas was effective in eliminating naturally occurring bacteria on animal skin and also on skin inoculated with *Bacillus* spores. The culturable bacteria, including *Bacillus* spores, were eliminated in a time- and dose-dependent manner. Results of these studies suggested portable, easily used ClO_2 gas generation systems have excellent potential for protocol development to contribute to biodefense strategies and decontamination responses to infectious disease outbreaks or other biothreat events.

Chlorine dioxide (ClO_2) in solution is a well-known disinfectant and is used in potable water treatment, food sanitation activities, and also as a bleaching agent in the pulp and paper industry.[1-3] Chlorine dioxide gas is likewise effective in disinfection applications.[4-7] The gas is also approved for anthrax spore decontamination and was used in response to the intentional release of *Bacillus anthracis* spores in the United States in 2001.[8,9]

More widespread use of ClO_2 gas for disinfection activities has been hampered because the gas is too unstable for shipment, and thus it must be prepared at the application site. Typically, this entails the use of dedicated equipment and trained personnel. Technology now exists that enables local ClO_2 gas to be generated without dedicated equipment, electricity, water, or personnel with advanced training. This affords the opportunity for ClO_2 gas applications that previously were nearly impossible or impractical to implement.

This study aimed to demonstrate the antibacterial efficacy of portable ClO_2 gas generation technology on model surfaces such as personal protective equipment (PPE) and animal hides. Objective achievement would advocate novel ClO_2 gas applications that could be relevant to biodefense needs, such as in a mass casualty event. These new systems can have a shelf life of several years and are highly portable. One such system consists of 2 granular components that

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when mixed together release ClO_2 gas in a predictable manner. The ClO_2 gas can also be mixed in a gas-permeable sachet and placed in water to create ClO_2 in solution. Previous studies from this laboratory have demonstrated the antibacterial properties of ClO_2 gas generated by one of these systems.[10,11]

The lack of specialized equipment needs, in conjunction with ease of use of this technology, have supported novel disinfection applications. For example, previous studies demonstrated ClO_2 gas efficacy in disinfecting sports equipment such as protective football pads.[11] The gas was also effective in inactivating *Bacillus atrophaeus* spores on a variety of materials including wood, glass, metal, and concrete.[12]

It is desirable to develop decontamination methodologies and strategies in response to biological incidents that are naturally occurring or the result of intentional human action.[13] Easily used, highly portable, granular component ClO_2 gas–generating systems could easily produce appropriate amounts of ClO_2 gas to support biodefense responses in a number of different arenas. The purpose of this investigation was to document ClO_2 gas antibacterial efficacy against novel material surfaces that could serve as a reservoir for bacteria harmful to human health.

Two concerns in disaster or biothreat efforts are the protection of first responders and mitigating the risk of handling potentially infective materials during disposal. It has been suggested that contaminated PPE could transfer infectious agents to PPE users.[14] The gear may not be easily sanitized and could be susceptible to contamination with human pathogens by users. For example, environmental surfaces can harbor methicillin-resistant *Staphylococcus aureus* (MRSA) and other pathogens.[15-18] An initial purpose of this investigation was to assess a highly portable ClO_2 gas–generation system in eliminating naturally occurring bacteria and laboratory-applied MRSA on MSA Ultra Elite® Responder® facepieces. PPE such as facemasks also have potential to be contaminated during use in response to biological agents such as anthrax. Therefore, the potential to inactivate *Bacillus* spores during mask treatment with ClO_2 gas was also assessed.

Natural disasters as well as bioterrorism events have the potential to produce human and animal casualties. These present challenges to remediation in scenarios where clean water and electricity may have temporary disruptions. Because skin is porous and hair-covered, its surfaces are particularly challenging to decontaminate. An additional goal of this study was to determine the effectiveness of a portable ClO_2 gas–generation system to eliminate vegetative bacteria and *Bacillus* spores on animal (swine) skin as proof of concept for carcass surface decontamination in a broad-scale mass casualty event. Elimination of surface bacteria has the potential to reduce exposure and risk that might be associated with handling animal skin products and animal casualties as the result of infectious agents. In an animal mass casualty event, handling of carcasses before disposal could pose a risk to humans and other animals. Although current technology for animal surface decontamination is typically viewed in terms of promoting food safety, decontamination of carcass surface tissue may be appropriate in a biothreat response to reduce pathogen exposure. An objective of this study was to evaluate the ability of ClO_2 gas to eliminate bacteria on carcass surface tissues in the context of its potential to reduce human exposure to pathogens in a biothreat response.

Materials and Methods

Portable ClO_2 Gas Generation and Antibacterial Efficacy on PPE

An initial aim was to determine if used PPE (Ultra Elite® Responder® facepiece) harbor bacteria that could be readily recovered and subsequently serve as a model for determining ClO_2 gas antibacterial efficacy. For recovery of naturally occurring bacteria, a wetted cotton swab was rubbed over the test site area (2.5 cm × 2.5 cm) and placed into a tube containing 10-ml phosphate-buffered saline (PBS) and agitated. A total of 11 different sites, representing internal and external surfaces, were sampled. Tenfold serial dilutions were plated in duplicate onto trypticase soy agar (TSA), which is a general-purpose medium that supports the growth of a large assortment of bacteria. Plates were incubated for 24 h at 37°C. All ClO_2 gas treatment efficacy studies were performed at room temperature (22°C), and the test areas consisted of facial contact points and the cloth head strap on Mine Safety Appliances (MSA) Company (Cranberry Township, PA) Ultra Elite® Responder® facepieces (Figure 1). Methicillin-resistant *Staphylococcus aureus* (MRSA) was then applied to the test areas. The MRSA (ATCC 43300) was cultured 12 h at 37°C in trypticase soy broth (TSB), harvested by centrifugation (2,000 × g), and diluted in PBS to 10^9 cells/ml. Ten μl of the suspension (containing 10^7 MRSA) was applied to test areas (2.5 cm × 25 cm each) and allowed to dry 30 min before treatment and recovery of culturable bacteria cells as previously outlined. Inoculation and recovery tests showed that 10^4 cells could typically be recovered from an untreated site. To document any sporicidal activity of mask treatments, biological indicators (MesaLabs, Bozeman, MT) inoculated with 10^3 spores of *Bacillus atrophaeus* were placed alongside the mask during ClO_2 gas treatments. After treatments the indicators were placed in 9 ml of trypticase soy broth (TSB) for 48 h at 37°C to determine outgrowth and spore viability.

Chlorine Dioxide Gas Treatments of Facepieces

Adjacent sites on facepieces were inoculated with MRSA as described previously. One inoculated site from each test site was sampled for recovery of MRSA before treatment with ClO_2 gas. The inoculated mask was then placed in an

Figure 1. Image of the MSA Ultra Elite® Responder® facepieces used in ClO_2 gas treatment studies

Figure 2. ClO_2 Clave unit designed for ClO_2 gas treatment studies

apparatus (termed a ClO_2Clave Unit, ICA TriNova, LLC, Newnan, GA) designed for ClO_2 gas treatment studies (Figure 2). The treatment chamber volume was 15 liters, and the apparatus was capable of measuring ClO_2 gas concentration from 0-9,000 ppm and relative humidity (RH) from 0-100%. Chlorine dioxide gas was prepared using the ICA TriNova (Newnan, GA) Z-Series™ granulated fast release generation system according to the manufacturer's instructions. The 2 granular components were mixed in ratios to produce the desired ClO_2 gas concentrations. The components were mixed in a gas permeable sachet and placed into the ClO_2Clave treatment chamber. To gauge the impact of RH on treatment efficacy, treatments were performed with and without addition of humidity chips (water impregnated cellulose pads, Andersen Sterilizers, Inc., Haw River, NC). After ClO_2 gas treatments, the gas was suctioned through an adjacent filter designed to remove and inactivate residual gas. The ClO_2Clave treatment chamber tests were performed in a laboratory room designed to remove undesirable fumes. If facepieces were treated more than once in efficacy studies, the test areas of the facepiece were thoroughly cleaned and allowed to dry at least 24 h prior to treatment. At least 2 observations were made for efficacy studies. Following treatments, visual inspections were performed to determine if repeated ClO_2 gas treatments might affect the physical condition of the facepiece.

Following decontamination efficacy studies, new MSA Ultra Elite® Responder® facepieces were exposed to 2 treatment protocols to simulate field conditions where ClO_2 gas might be used to meet potential routine sanitation needs as well as a more robust decontamination relevant to spore deactivation. To demonstrate potential for use in austere or field-based conditions, a sachet capable of generating the ClO_2 gas was placed adjacent to a new facepiece in a 30-liter plastic bag purchased from a local retail outlet. To simulate the potential routine sanitation use, the mask was treated 6 times with approximately 100 ppm of ClO_2 gas for 1 h. To simulate a less-frequent but more robust decontamination use, a second new mask was twice treated similarly but subjected to approximately 1,000 ppm ClO_2 gas treatments. This higher-level treatment might not be considered for a routine procedure, as it also has greater potential to affect the physical and functional integrity of the facepiece. A level approaching 1,000 ppm would most likely be considered in a rare event where killing of bacteria spores (such as *B. anthracis*) is a desired outcome. Plastic treatment bags were opened outdoors to vent any residual gas. Following treatments, masks were examined for readily visible changes that could affect function. Biological indicators (spore strips containing 10^3 *B. atrophaeus*) were included in the gas treatments and assessed for posttreatment outgrowth in TSB.

Portable ClO_2 Gas Generation and Antibacterial Efficacy on Swine Skin

Swine skin was purchased from a local pork processing facility. Untreated swine skin, with hair intact, was

Table 1. Recovery of naturally occurring bacteria colony forming units (CFU) from test sites (2.5 cm × 2.5 cm each) on a used MSA Ultra Elite® Responder® facepiece

Test Site	*Log_{10} CFUs Recovered*
Filter outside mask	2.55
Filter inside mask	3.24
Shield inside mask	4.20
Face contact area forehead	3.59
Face contact area chin	3.47
Face contact area left side	3.39
Face contact area right side	3.29
Face contact area top nose	3.64
Lower nose area (noncontact area)	4.18
Cloth strap attached to mask	5.27
Mesh covering (fits over head)	5.09

removed at the pork processing facility. Collectively, the surface skin, underlying fascia, and fat layer was approximately 1.0 cm thick. Bacteria efficacy studies were performed only on the skin surface. Use of swine skin for this study was conducted according to the Middle Tennessee State University Institutional Animal Care and Use Committee Policy on use of animal products from commercial vendors. For laboratory studies, skin was cut into pieces measuring approximately 5 × 5 cm and stored at −20°C or 4°C. Prior to use, skin was allowed to come to room temperature. In spore studies, skin was inoculated with 10 μl of a suspension containing 4.5×10^7 spores of *Bacillus atrophaeus* (MesaLabs, Bozeman, MT). This bacterium has been used as a surrogate in anthrax decontamination studies. Culturable bacteria were recovered from skin surfaces by rubbing cotton swabs over the skin surface and then onto TSA plates. In some instances (and for comparative purposes), the skin surface was sampled directly by use of contact plates filled with TSA. Here the surface of the agar extends above the lip of the petri dish. The plates are then gently pressed directly against the surface to be sampled for recoverable bacteria. Contact plates have a history of use for sampling surfaces. Trypticase soy agar plates inoculated with test samples were incubated at 37°C for 24 h and subsequently incubated an additional 24 h at 22°C to recover naturally occurring bacteria colony forming units (CFU) that might not multiply well at 37°C. For ClO_2 treatments of skin, the gas was generated as previously described. Chlorine dioxide treatment studies of swine skin were also conducted in the ClO_2Clave, as it provided a more controlled environment along with measurements of ClO_2 gas concentration and RH. Commercially available biological indicators (MesaLabs, Bozeman, MT) inoculated with 10^4 and 10^6 spores of *B. atrophaeus* were also included in skin decontamination studies. These were subsequently incubated at 37°C for 48 h to assess posttreatment spore viability.

Results

Recovery of Bacteria from Treated and Untreated Facepieces

Naturally occurring bacteria were readily recovered from an MSA Ultra Elite® Responder® respirator facepiece that was used on a regular basis and not in an established cleaning program (Table 1). Colony-forming units were recovered from all the hard surfaces, the mask strap, and mesh head covering test areas on the mask. The greatest number of naturally occurring recoverable CFU were associated with the cloth strap and mesh that fits on top of the head (Table 1). Duplicate test sites on the mask were inoculated with 10^7 CFU of MRSA and allowed to dry before pre- and posttreatment recovery of CFU. After bacteria were recovered from the pretreatment sites, the facepiece was immediately treated with ClO_2 gas, and then the bacteria were recovered from the adjacent posttreatment sites.

For the mask, the experimental design was composed of 3 treatment times (1, 1.5, and 3 h), 2 humidity levels (approximately 40% and 60%), and 2 ClO_2 gas treatment levels (approximately 140 and 190 ppm). In all facepiece ClO_2 gas treatments, recoverable bacteria after treatment (including MRSA inoculated onto mask sites) was reduced by more than 2 logs, a 99% or greater reduction (Table 2). This reduction was a feature of the shortest treatment time evaluated (1 h) in conjunction with the lower RH level and

Table 2. Average recovery of bacteria colony forming units (CFU) before and after ClO_2 gas treatments from MSA Ultra Elite® Responder® facepiece inoculated with methicillin-resistant *Staphylococcus aureus*

Treatment Time (hours)	*Maximum Chlorine Dioxide Concentration Achieved (ppm)*	*Maximum Relative Humidity Achieved (%)*	*Average Log_{10} CFUs Recovered Before Treatment*	*Average Log_{10} CFUs Recovered After Treatment*	*Log_{10} CFU Reduction*
1	140	44	4.45	2.30	2.15
1	150	66[a]	4.41	0	4.41
3	125	48	4.78	1.45	3.33
3	140	64[a]	4.52	0	4.52
1.5	190	47	4.04	1.79	2.25
1.5	200	65[a]	4.70	0	4.70

[a]Humidity chips added.

Table 3. Average recovery of bacteria colony forming units (CFU) before and after ClO_2 gas treatments from MSA Ultra Elite® Responder® facepiece strap inoculated with methicillin-resistant *Staphylococcus aureus*

Treatment Time (hours)	*Maximum Chlorine Dioxide Concentration Achieved (ppm)*	*Maximum Relative Humidity Achieved (%)*	*Average Log_{10} CFUs Recovered Before Treatment*	*Average Log_{10} CFUs Recovered After Treatment*	*Log_{10} CFU Reduction*
1	140	44	4.28	1.41	2.87
1	150	66[a]	3.04	0	3.04
3	125	48	4.45	1.15	3.30
3	140	64[a]	3.30	0.78	2.52
1.5	190	47	4.23	0	4.23
1.5	200	65[a]	3.32	0	3.32

[a]Humidity chips added.

ppm (140 ppm) gas concentration. Increasing ClO_2 gas ppm and treatment time increased efficacy of treatment. Increased RH also enhanced efficacy, as recoverable CFU were reduced to <1 at RH $>60\%$ (Table 2).

A greater number of naturally occurring bacteria was initially recovered from the mask strap and mesh head covering (Table 1). MRSA was likewise inoculated (10^7 CFU) onto these fittings. Treatment times, ppm of ClO_2 gas, and RH parameters were comparable to those used in the treatment of the mask hard surfaces. Reduction of recoverable CFU was comparable to that observed with the mask hard surface treatments in that recoverable CFU was reduced by greater than 2 logs. An average recoverable CFU to less than 1 was achieved with increasing the ppm of ClO_2 to 190 ppm and the RH to above 60% (Table 3).

Two new MSA Ultra Elite® Responder® facepieces were also subjected to visual examination and inspections following treatments with ClO_2 gas to simulate field conditions where ClO_2 gas might be used to meet potential routine sanitation needs as well as a more robust decontamination. To simulate a routine sanitation treatment, a new mask was treated 6 times at approximately 100 ppm for 1 h in a garbage bag. To simulate a less frequent, but more robust treatment, another mask was likewise treated twice at 1,000 ppm ClO_2 gas for 1 h. To confirm the efficacy of the more robust treatment (1,000 ppm ClO_2 gas), biological indicators (spore strips containing 10^3 *B. atrophaeus*) were included in the gas treatments. Sporicidal activity of this treatment protocol was confirmed based on the lack of outgrowth of biological indicators following treatment. Growth was observed with all untreated (negative) control biological indicators. After these treatments were performed, there was no apparent change in the physical condition of either facepiece.

Recovery of Bacteria and Spores on Swine Skin Before and After Treatment with ClO_2 Gas

When spores of *B. atrophaeus* were inoculated onto swine skin, the average number of recoverable naturally occurring bacteria plus the spores was up to 7 logs before treatment with ClO_2 gas (Table 4). This was 3 to 4 logs greater than that observed with the facepieces (Table 3). To achieve greater than 3 log (99.9%) reduction of bacteria (including spores) on the swine skin, the treatment times (up to 6 h) and concentration (up to 3,000 ppm) of ClO_2 gas in ppm was greatly increased (Table 4). In addition to using a cotton swab suspended in PBS to establish recoverable bacteria as CFU, the use of contact plates also demonstrated the ClO_2 gas treatments' ability to considerably diminish the recovery of bacteria (Figure 3). In all the ClO_2 gas treatments performed, greater than 3 logs (99.9%) of recoverable CFU were eliminated (Table 4). A treatment time of 2 h at 1,100 and 2,700 ppm ClO_2 gas was effective in eliminating recoverable CFU but failed to eliminate all culturable spore-forming *B. atrophaeus*. However, the inactivation of *B. atrophaeus* spores that were applied to the skin was a feature of certain gas treatments. The elimination of naturally occurring bacteria and spore outgrowth was observed only at the longest treatment time investigated (6 h). This was apparent based on recoverable CFU from

Table 4. Recovery of viable spores of *Bacillus atrophaeus* and naturally occurring bacteria from swine skin before and after treatment with ClO_2 gas

Treatment Time (hours)	*Maximum Chlorine Dioxide Concentration Achieved (ppm)*	*Average Log_{10} CFUs Recovered Before Treatment*	*Average Log_{10} CFUs Recovered After Treatment*	*Log_{10} CFU Reduction*
2	1,110	7.09	3.18	3.91
2	2,760	6.83	1.38	5.45
4	3,035	6.94	0	6.94
6	560	7.04	1.81	5.23
6	1,450	7.03	0	7.03
6	3,070	7.31	0	7.31

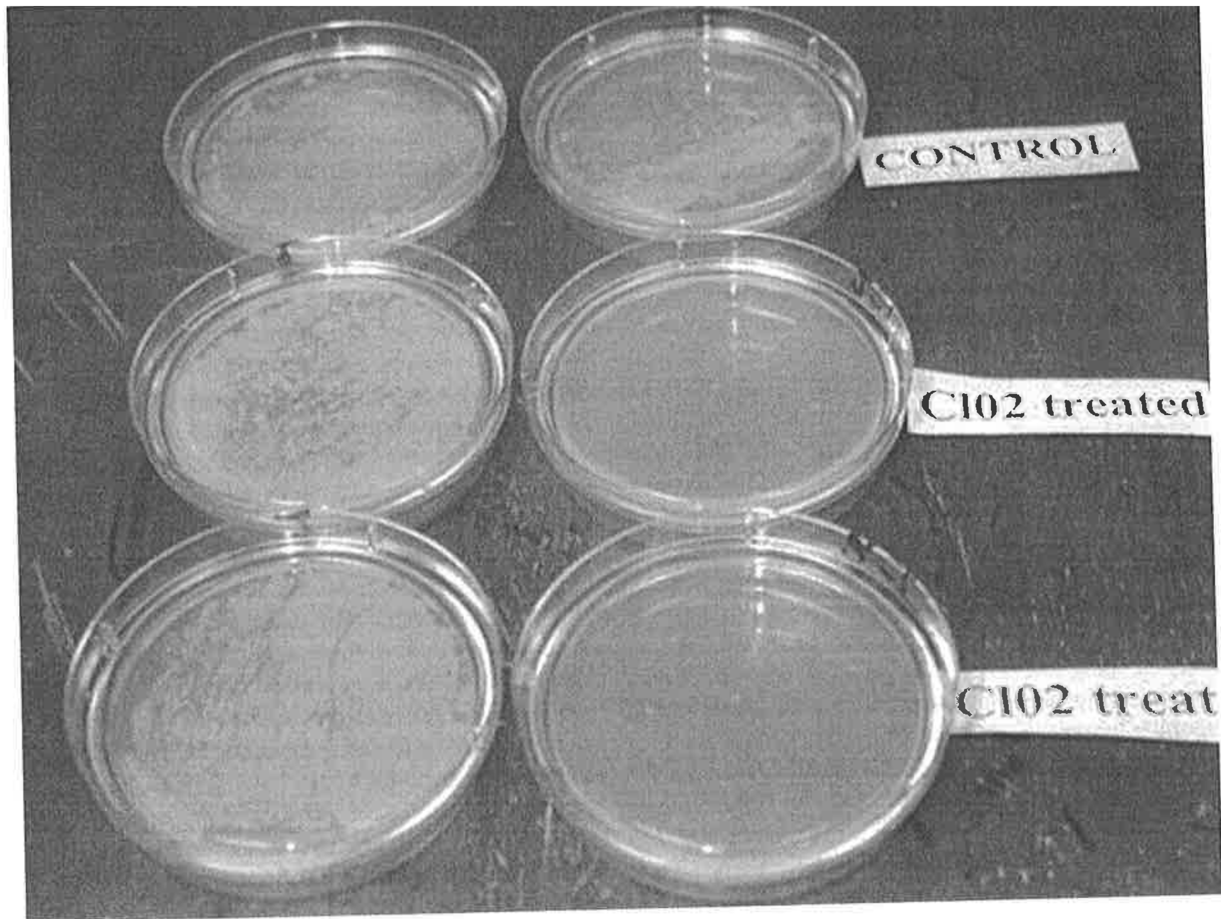

Figure 3. Use of contact plates demonstrated reduction or elimination of culturable bacteria from swine skin following treatment with ClO_2 gas.

cotton swab samples and from the use of contact plates (Figure 4). If the ppm of ClO_2 gas was lowered to 550 ppm in the 6-h treatment, naturally occurring bacteria were effectively eliminated, but spores of *B. atrophaeus* inoculated onto the skin and naturally occurring bacteria were not entirely eliminated.

Biological indicators composed of 10^4 and 10^6 *B. atrophaeus* were also included with the ClO_2 gas treatments of swine skin. No outgrowth of spores occurred from the 10^4 biological indicators in any of the gas treatments. Outgrowth of spores did occur after the 2-h treatment using the 10^6 *B. atrophaeus* biological indicators. When treatment

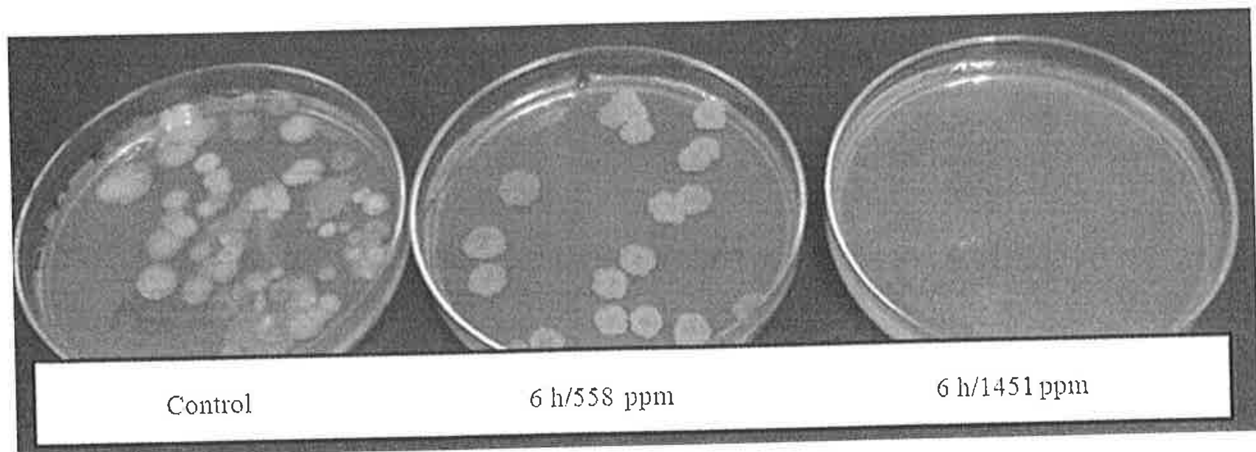

Figure 4. Outgrowth of bacteria recovered from swine skin inoculated with *Bacillus atrophaeus* spores following 6 h treatment with 558 and 1,451 ppm ClO_2 gas

times were increased to 4 h and 6 h, the growth from 10^6 biological indicators could be eliminated. However, if the ppm of ClO_2 gas was 550 ppm, even the 6-h treatment did not eliminate outgrowth of spores from the 10^6 biological indicators.

Discussion

Portable, easily used ClO_2 gas–generation systems could be used to support approaches to reduce or eliminate undesirable bacteria associated with a variety of environmental surfaces relevant to biodefense strategies. The ability to recover 10^2 to 10^5 log of naturally occurring bacteria from test sites on a used MSA Ultra Elite® Responder® facepiece suggested that masks can serve as a reservoir for infectious bacteria. A much greater number of recoverable bacteria were associated with the more porous head strap and mesh covering for the head, and this should also be included in any mask sanitization treatment protocol.

A purpose of the current study was to investigate the efficacy of using low-dose ClO_2 gas treatments for eliminating naturally occurring bacteria on used Ultra Elite® Responder® facepieces and also for MRSA when applied to the facepieces. Laboratory tests demonstrated bacteria will survive on hard and soft facepiece surfaces and on facepiece porous materials. This supports the idea that PPE has the potential to serve as a reservoir for infectious agents. Test results demonstrated that ClO_2 gas was easily used and effective in reducing naturally occurring recoverable bacteria associated with facepiece surfaces and the more porous head strap and mesh covering. When MRSA was applied to mask test sites, multiple log reductions in MRSA recovery following ClO_2 gas treatments was also demonstrated. Efficacy of bacteria load reductions were documented on the rubber contact points, the porous cloth strap, and the mesh covering. Typically, for most treatment conditions, a 3-log or greater reduction was achieved. The purpose of bacteria decontamination is to reduce the number of microorganisms to safe public health levels. As demonstrated here, the elimination of recoverable bacteria in some instances suggested PPE could be decontaminated by treatment using a portable ClO_2 gas–generation system. The elimination of only a portion of the bacteria (log reduction) is less supportive. In the food preparation industry, a mandated 5-log reduction of acid-resistant bacterial pathogens (including *Escherichia coli* O157:H7) is in place.[19] However, there is an absence of standards for decontamination of PPE that are likewise directly linked to the number of log reductions of bacteria. If ClO_2 gas treatments are developed as part of PPE decontamination protocols, further studies would also be needed to be certain the treatments do not have an impact on physical condition or function. To promote use, any treatment program should be easily applied and effective in eliminating bacteria. Results suggested the use of ClO_2 gas would be useful in developing field-based strategies for eliminating bacteria from protective facepieces and other PPE attire among users. Future studies should also address any impact the ClO_2 gas treatments might have on subsequent mask function.

Historically, the quantification of the normal flora of swine skin has not been the objective of scientific examination.[20] It likewise was not the objective of this study. A recent study, however, has examined the flora of swine skin and shown a broad spectrum of bacteria can be isolated.[20] Results of the current study suggested a role for ClO_2 gas in decontaminating animal surface tissue (skin).

Disease outbreaks resulting in the death of domestic animals can be naturally occurring events, but there is also the potential for the deliberate initiation of a disease outbreak. In response to this, decontamination strategies that require no dedicated equipment, electricity, water, or highly trained personnel are highly relevant to a biothreat or animal mass casualty response. The results of the current study suggested a potential role for ClO_2 gas in biothreat mitigation activities.[21] For example, in an animal mass casualty event, makeshift treatment enclosures of plastic or other materials may be employed to meet immediate, short-term infectious risk reduction needs, thus bridging a critical gap between short-term response and what might become a delayed, final disposition.

In addition to bioterrorism and biodefense strategy needs, there are also commercial animal skin decontamination needs. Animal skin can be contaminated with naturally occurring human pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, or *Escherichia coli* 0157:H7.[22,23] Bacterial load and cleanliness of superficial hide surface may also play a role in bacterial numbers on meat products.[23,24] When live animals are processed to consumable meat products, there invariably exists potential for transfer of pathogenic bacteria to the meat products; a food safety principle is to minimize transfer of carcass bacteria during food preparation. Examples of some current practices include a hot water rinse and a lactic or acetic acid rinse.[25]

In the current study, the ability of ClO_2 gas to dramatically reduce or in some instances eliminate the bacteria load, including spores associated with skin from a swine carcass, suggested it could provide an additional opportunity as an intervention strategy in naturally occurring events or the result of bioterrorism. Currently used animal processing technology would not be expected to eliminate bacteria spores associated with animal skin. Although elevated levels of ClO_2 gas were necessary to effectively reduce spore-forming bacteria inoculated onto the skin, it does demonstrate that technology is available to reduce and eliminate spores if that is a desired endpoint of treatment processing. In related studies we observed that ClO_2 solutions were much less effective than the gas in eliminating bacteria on animal skin (data not shown). Comparative studies have also suggested that gaseous ClO_2 treatments are more effective in eliminating surface bacteria on plant tissue than washing or

using disinfection solutions.[26] It is likely that gas may be more efficient than liquid-based antimicrobials in penetrating porous material. It was apparent in the current study that reduction and elimination of bacteria on the organic substrate (animal skin) required a more vigorous treatment than that observed with the facemasks. This could relate to the porous property of skin, the protective effect provided by hair on the skin, or the more rapid consumption of ClO_2 gas by the organic pig skin.

It is likely that urban wide recovery needs after a large-scale intentional biological release will exceed the capacity of administrative authorities and private contractors. It has been suggested that a need exists to develop biological remediation processes for use by the private sector, which oversee much of the nation's critical infrastructure facilities.[27] This suggests a potential role for the owner/occupant to participate in remediation activities. In addition, ClO_2 gas is also currently considered to be a viable choice for fumigating large indoor areas.[28] Chlorine dioxide gas generation technologies such as those demonstrated in this study are currently in use by owners and workers in the private sector and could be used to support essential needs in response to a bioterrorism event.

Performing laboratory studies is a necessary initial step in developing disinfection technologies to meet biodefense strategies for a variety of current and possibly unforeseen future needs. The results of this study provide new awareness and insight into sanitization and decontamination possibilities. Portable, easily used ClO_2 gas–generation systems, such as the type used in this investigation, could be used to support development of easily used, field-based decontamination protocols. All-inclusive, specific protocols will require additional studies to validate ClO_2 gas as an intervention strategy and optimize parameters for broad-scale use.

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