

Introduction

The ability to timely and safely contain the spread of bacterial pathogens in an outbreak is a matter of high concern for national security as well as the national food supply.

In this study, chlorine dioxide (ClO₂) was evaluated as a means of decontaminating animal carcasses to decrease the risks involved in handling and disposing of mass casualties that have been contaminated with both naturally-occurring pathogens, as well as spore-forming bacterial pathogens that might be used in a deliberate terrorist attack or in a naturally-occurring outbreak.

The use of chlorine dioxide as a decontaminant for anthrax and other biological agents is not new. (1) It is used worldwide for the treatment of potable water, in food preparation and for bleaching pulp in the paper industry. (2, 3) Several studies involving the use of chlorine dioxide as a decontaminant have been conducted at Middle Tennessee State University including treatment of sports equipment and building materials. (4, 5) Chlorine dioxide was also used in efforts to decontaminate federal office buildings following the intentional release of anthrax spores (in mail) in 2001. (6)

The gas is too unstable to be shipped and must be prepared at the application site. Issues related to the safety of transport and expertise needed to generate it on-site have hindered its broad-scale consideration in local response scenarios. The recent development of a two-component sachet delivery system eliminates these problems, making it prudent to evaluate potential use in local response scenarios.

Materials and methods

Chlorine dioxide gas was generated by combining equal parts of a two component generation system (ICATriNova, Newnan, GA) in a water-resistant, gas-permeable sachet. ClO₂ solutions were prepared by immersing a sachet in deionized water and water from a local stream.



Figure 1. ClO₂ generating components mixed in a sachet



Figure 2. ClO₂Clave Unit (ICATriNova, Newnan, GA)

Gas treatments were accomplished in containers that were available at local retailers including trash bags and plastic containers. The ClO₂Clave device provided by ICATriNova, was used to provide a controlled test environment and measure chlorine dioxide gas concentrations in parts per million (ppm) and relative humidity during the test period.



Figure 3. Inoculation of skin samples with spores of *Bacillus atrophaeus* (Apex Laboratories, Apex, NC)

Untreated pig skin samples were inoculated with 10ul of a suspension containing 4.5x10⁷ spores of *Bacillus atrophaeus*, a spore-forming bacterium that is commonly used as a model for anthrax decontamination studies. Skin samples were then treated with chlorine dioxide gas and solutions.

Treated and control skin surfaces were analyzed using swabs and contact plates. Due to naturally-occurring skin bacteria and the added spore suspension, untreated samples were plated on trypticase soy agar (TSA) using serial 10-fold serial dilutions to obtain usable plate counts. Treated samples were transferred directly from swabs to TSA plates or by direct sampling using TSA contact press plates. All plates were incubated at 37C for 24 hours plus an additional 24 hours at 22C. Agar plates were counted to determine colony forming units (CFUs) and treatment efficacy.

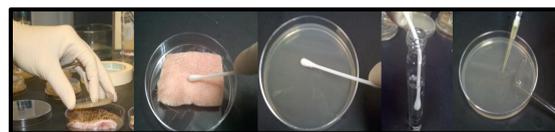


Figure 4. Skin sampling and plating



Figure 5. Treated spore strips were added to TSB to determine viability

Spore strips of *B. atrophaeus* are commonly used in determining the effectiveness of sterilizing protocols such as autoclaving. Spore strips containing 10⁴ and 10⁶ spores of *B. atrophaeus* (Apex Laboratories, Apex, NC) were treated with chlorine dioxide gas and incubated in trypticase soy broth (TSB) at 37C for 24 hours to determine post-treatment viability.

Results

Table 1. Chlorine dioxide gas treatment results

Treatment Time	Mass of Each Reactant (grams)	ClO ₂ Maximum (ppm)	Chlorine Dioxide Gas Treatment Results			
			Untreated Control Samples	Treated Samples ⁽¹⁾	Change	Percent Reduction ⁽²⁾
2 Hours	10	1,109	12,250,000	1,524	-12,248,476	99.9+%
2 Hours	20	2,760	6,825,000	24	-6,824,976	99.9+%
4 Hours	20	3,035	8,700,000	1	-8,700,000	99.9+%
6 Hours	5	558	10,950,000	64	-10,949,936	99.9+%
6 Hours	10	1,451	10,850,000	0	-10,850,000	100%
6 Hours	20	3,067	20,250,000	0	-20,250,000	100%

(1) There was no growth on any replicate of samples indicating zero CFUs. (2) Although replicates confirmed these results, sampling methods are not 100% effective in recovering all bacteria from a surface or transferring all recovered bacteria to a growth medium.



Figure 6. Results of gas treatment on *B. atrophaeus* 10⁶ spore strips

Table 2. Chlorine dioxide gas treatment effect on *B. atrophaeus* spore strips

Growth from *Bacillus atrophaeus* Spore Strips Following Treatment*

Treatment Time	Mass of Each Reactant (grams)	ClO ₂ Maximum (ppm)	10 ⁴	10 ⁶
2 Hours	10	1,109	Negative	Postive
2 Hours	20	2,760	Negative	Postive
4 Hours	20	3,035	Negative	Negative
6 Hours	5	558	Negative	Positive
6 Hours	10	1,451	Negative	Negative
6 Hours	20	3,067	Negative	Negative

*There was negative growth on all replicate of samples where a negative result is indicated. Strips were incubated in TSB at 37C for 24 hours.



Figure 7. Plates showing reduction of bacteria after treatment with chlorine dioxide solution

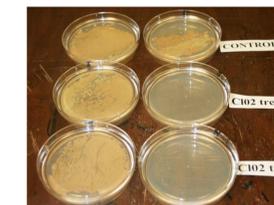


Figure 8. Plates showing elimination of naturally-occurring skin bacteria and added spores using chlorine dioxide gas treatment

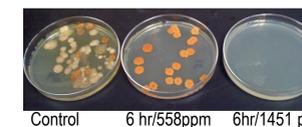


Figure 9. Doses that are effective in inhibiting some bacteria do not eliminate spore-formers. A 6 hour treatment at 558 ppm was successful in eliminating bacteria that are not spore-formers, but did not eliminate *B. atrophaeus* (large orange colonies left and center.) However, a 6 hour treatment with a higher concentration (1451 ppm) was effective in eliminating all recoverable bacteria.

Conclusions

Results showed that chlorine dioxide gas was effective in eliminating naturally-occurring skin bacteria as well as the spore-former *B. atrophaeus* inoculated onto pig skin. Spray and dip treatments utilizing chlorine dioxide solutions were effective in eliminating a portion of naturally-occurring skin bacteria, but not effective in eliminating *B. atrophaeus* spores.

Effective gas dosage is mass, time and concentration dependent. In these studies, treatments that achieved a maximum concentration of at least 1,451 ppm for 6 hours were effective in eliminating all spores and naturally-occurring skin bacteria.

Skin is a unique surface to decontaminate and treatment protocols that were successful in eliminating spore-forming bacteria on spore strips were not equally effective on skin surfaces (Tables 1 and 2.)

Gas treatments were generally more effective when spores were added more than one hour prior to treatment. This suggests skin may activate spores making them more susceptible to treatment. (These results were excluded from data presented in Table 1.)

There are clear applications for the use of chlorine dioxide in infectious outbreak responses to mitigate exposure risks in the handling and disposal of animal mass casualties. Additional research is needed to optimize broad-scale application protocols for use in responding to a naturally-occurring outbreak or deliberate terrorist attack.

Literature cited

- Hass C. Decontamination using chlorine dioxide [Internet]. Washington, DC. Committee on science and technology; 2001. Available from www.science.house.gov.
- Ieta, E; and Berg, J. 1986. A review of chlorine dioxide in drinking water treatment. Water Works Assoc. 78:62-71.
- Simpson, GD; Miller, RF; Laxton, GD; and Clements, WR. A focus on chlorine dioxide: the ideal biocide, paper No 472, Corrosion 93, New Orleans, LA., (March 8-12, 1993).
- Newsome, AL; DuBois JD; and Tenney, JD. 2009. Disinfection of football protective equipment using chlorine dioxide produced by the ICA system. BMC Public Health. 9:326-334.
- Salehzadeh, I, Decontamination assessment of *Bacillus atrophaeus* spores on common surfaces using chlorine dioxide gas and a novel device (MS Thesis) Murfreesboro, TN: Middle Tennessee State University. 2008.
- Hearing before the Committee on Science, House of Representatives. The decontamination of anthrax and other biological agents [Internet]. Washington, DC. Committee on Science and Technology; 2002. Available from www.science.house.gov.

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For further information