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The sporicidal potency of bioxy formulations in decontaminating bio-warfare agents

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Abstract

Biological terrorism has become a major concern worldwide since the 2001 mailing of anthrax spore-tainted letters. *Bacillus globigii* has been used as one of surrogates for the pathogenic *Bacillus anthracis*, the causative agent of anthrax. Many chemical compounds have been used to decontaminate anthrax spores such as chlorine, peroxide, N-chloro derivatives of ammonia, and chlorine dioxide. However, some of these compounds are not environmentally-friendly, as they are either corrosive to surfaces or toxic to humans. We propose Bioxy formulations which are powdered concentrated products that generate peracetic acid at near-neutral pH when dissolved in water. Solutions derived from such formulations are very effective virucidal, bactericidal, fungicidal and tuberculocidal compounds. Moreover, they are safe on various metallic and polymeric surfaces with strong wetting and penetrating abilities. In this study, we demonstrate that a 2% concentration of "Bioxy Enviro" is a highly effective sporicidal formulation, since >5-6 logs reduction in the number of viable spores of *Bacillus globigii* within 10- 30-min exposure was observed. Furthermore, when DeconGel was reformulated with Bioxy solution at 2%, the waste generation was minimized and the decontamination in hard to reach places was maximized, since >5-6 log spore kill of *Bacillus anthracis* was observed on four surfaces.

Introduction

Biological terrorism has become a major concern not only in the United States but also worldwide, since mailing of the anthrax spore-tainted letters in the fall of 2001 which resulted in the contamination and closure of the U.S. Postal Service Curseen-Morris Processing and Distribution Center (Brentwood Post Office) [1]. Following a covert release of spores of *B. anthracis*, it is largely understood that decontamination and cleanup of infrastructure interior and exterior will likely consume the Nation's remediation capacity, requiring years to cleanup, and leading to incalculable economic losses [2-4].

Thus, awareness and disaster preparedness for biological terrorism have become a priority in North America to limit the adverse impact of the biological pathogens in the event of a wide area release and contamination in an outdoor or subterranean environment.

Because a bioterrorism event is a disaster that requires specific preparations beyond the usual medical disaster planning; an effective response therefore should include attention to infection control issues as well as plans for antimicrobial prophylaxis. This will largely impact not only the general population but also Health care facilities.

Since the impact of an infectious disaster will most likely be overwhelming to emergency departments, a collaboration between emergency and infectious disease specialists is critical in developing an effective response [5].

Bacillus globigii has been extensively used as a model simulant for *B. anthracis* spore [6,7]. This nonpathogenic bacteria is often used as a Biological Warfare simulant because it approximates the size, shape, and cellular organization of the pathogenic *B. anthracis*, the causative agent of anthrax [6,8].

Many chemical compounds have been used to decontaminate anthrax spores such as chlorine, the N-chloro derivatives of ammonia, methylamine, glycine and chlorine dioxide [1,9].

It is noteworthy to mention that halogens (ex. bromine, hypochlorite and iodophors), and aldehydes (ex. formaldehyde, glutaraldehyde and *o*-phthalaldehyde) have strong antibacterial properties; however, they have toxic and corrosive effects and thus cannot be fumigated because they irritate respiratory systems and corrode surfaces and equipments [10-17]. In addition, other inorganic compounds such as titanium dioxide, silver nitrate and zinc oxide [18-22] have also strong bactericidal properties against various pathogens but they persist in the environment and can be potentially toxic to humans and corrosive to surfaces. The aim of this study was to assess the sporicidal potency of Bioxy formulations against BW agents in order to propose to end-users a safer alternative to traditional disinfectants. The Bioxy formulations are powdered concentrated (patented) products that generate peracetic acid *in-situ* when dissolved in water at near-neutral to mild-alkaline pH levels. Bioxy formulations are effective against various viruses (including Ebola virus; unpublished results), bacteria, yeast and mold [10,23-25]. Unlike other disinfectants, Bioxy formulations are safe on various metallic and polymeric surfaces [26] with strong wetting and penetrating abilities [27].

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Materials and methods

Preparation and Test Method for *B. globigii* spores to determine the efficacy of "Bioxy Enviro" formulation

The efficacy study of "Bioxy Enviro" against *Bacillus globigii* spores was conducted using the AOAC 2008-05 modified method as described by Rastogi *et al*. [28].

Preparation of "Bioxy Enviro" solution

Two percent "Bioxy Enviro" solution was prepared by dissolving 20 grams "Bioxy Enviro" in 1L distilled water. This solution was mixed for 10 minutes at room temperature prior to application. The spores of *B. globigii* were exposed to the "Bioxy Enviro" 2% (w/v) solution for 10 and 30 min.

Re-formulation of DeconGel with Bioxy solution

The objective of this experiment was to develop a sporicidal hydrogel product based upon modifications to DeconGel to render the product efficacious for inactivation of spores of *B. anthracis* (ΔSterne) and related surrogate, *B. subtilis. B. anthracis* (ΔSterne) strain which is a non- pathogenic strain related to the pathogenic counter-part, Ames. DeconGel was modified via inclusion of sporicidal chemistry to achieve a kill of >5-6-log spores (spore kill). Three specific advantages of the proposed hydrogel technology are:

a. minimal waste generation (dried peeled film and no liquid waste).

b. minimal re-aerosolization (the spores are immediately locked in).

c. the hydrogel technology allows enhanced contact and resident time for the active ingredient to act on the spore surface permitting the use of reduced concentrations of sporicidal active ingredients.

Decontamination efficacy of Bioxy reformulated into DeconGel against *BaS* spores was evaluated on four selected surfaces: 1) concrete, 2) painted steel, 3) bare aluminum, and 4) glazed tile. The experiment was performed at Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Grounds, MD, in building E-3150. Aerosolized *BaS* spores were inoculated on pre-sterilized coupons of aluminum (A), painted steel (PS), concrete (C), and glazed Tile (T). The deposition density per coupon (10-sq-cm) was determined to be 6-7-logs per coupon. The percent recovery from coupons by base gel was determined to be >6-logs and accounted for 10-100% (depending on the surface type inoculated) of the inoculated spore numbers. The sporicidal efficacy of this formulation was evaluated immediately after or 2- and 4-hour after preparation. The log reduction values were >5-6-logs on all four surfaces. In summary, this formulation was highly effective (>5-6-log reduction) against spores of *BaS*. This experiment was repeated three times.

Preparation and application of Bioxy re-formulated into DeconGel

18.0-g of Bioxy were weighed and 31.7-ml of water were added. The solution was stirred with a magnetic stir bar for 1-hour with vigorous spinning. 180-g of hydrogel were stirred with an overhead stirrer at 1300-rpm. 6.0-g of glacial acetic acid were added. Bioxy solution was added slowly. When the solution came in contact with the hydrogel, foaming was observed. Then the solution was stirred for 10 minutes at 1300-rpm. Immediately the coupons were applied using a single use plastic pipette for ZERO time, and after 2 and 4 hours, on a set of 5 coupons for each surface type. This formulation comprises a hydrogel modified with Bioxy as the sporicidal active ingredient (6% by weight final concentration in the gel). The powder is first mixed with water and then added to the base gel and mixed for 10 minutes before use. The gel used in this study is modification of hydrogel 1128D, CBIP's sprayable/peelable coating, and effective in the decontamination of radiological and chemical contaminants. The base gel was provided to ECBC by CBIP, and the Bioxy was procured from Atomes F.D. Inc. The formulated gel was layered on a set of coupons immediately after mixing, and on another set of coupons 2 and 4 hours after mixing.

Results

Efficacy of "Bioxy Enviro" formulation against *B. globigii* spores

Within 10- and 30-min exposure, a 2% concentration of "Bioxy Enviro" demonstrated over 5-6-logs reduction in number of viable spores of *B. globigii* as outlined in Table 1. The results demonstrate that the Bioxy formulation is able to control the spores of *B. globigii*.

Sporicidal efficacy of Bioxy reformulated into DeconGel

The Log reduction values were all >5-6 for all material types. Clearly, the results show rapid and high effectiveness of the formulated gel against all four coupon types (Figure 1). Interestingly, the gel retains its effectiveness for a period of at least 4 hours, with no noticeable drop in efficacy. This demonstration is clearly an important milestone in present effort, since a gap of few hours between the formulation and actual application to the contaminated areas is inevitable. It is reassuring to note that no significant drop in the efficacy of the formulated gel is evident for the first four hours after preparation of Bioxy reformulated into DeconGel. The results demonstrate that the Bioxy formulated into DeconGel can solve an issue if outbreak occurs in hard to reach places such as subterranean environment (*i.e.,* subway station).

Discussion

The aim of this work was to characterize the efficacy of Bioxy against *B. globigii* and B. anthracis (ΔSterne), both of which are surrogates for the pathogenic *B. anthracis*, the causative agent of anthrax as well as to reformulate Bioxy into a DeconGel in order to minimize waste generation and maximize the decontamination especially in hard to reach places such as subterranean environment (*i.e.,* subway station).

We demonstrated that a 2% concentration of "Bioxy Enviro" resulted in 5-6-log reduction in number of viable spores of *B. globigii* within 10- and 30-min exposure time. Moreover, when Bioxy was reformulated into a DeconGel, the formulation was highly effective against spores of *B. anthracis* with >5-6-log reduction in number of viable spores. Further large-scale studies are essential to measure and monitor the efficacy of the Bioxy formulations on actual surfaces of hard to reach areas.

Table 1. Efficacy of 2% solution of "Bioxy Enviro" against spores *of Bacillus globigii*. The experiment was conducted in duplicate (Run 1 and 2). The mean Log-reduction of 10 minutes exposure to 2% solution (w/v) of Bioxy Enviro is 7.18-1.94 = 5.24.

	Log CFU of B. globigii Spores Recovered after 10 and 30 Minutes Exposure to 2% Bioxy-Enviro			
Run	**Log CFU recovered (Controls)**	**Log CFU recovered (Bioxy Enviro)**	**Log CFU recovered (Controls)**	**Log CFU recovered (Bioxy Enviro)**
	10 min		**30 min**	
Run 1	7.03	3.65	7.02	0.7
Run 2	7.33	0.23	7.21	1.2
Run 3	7.19	2.01	7.01	1.4
Mean	7.18	1.63	7.1	1.1

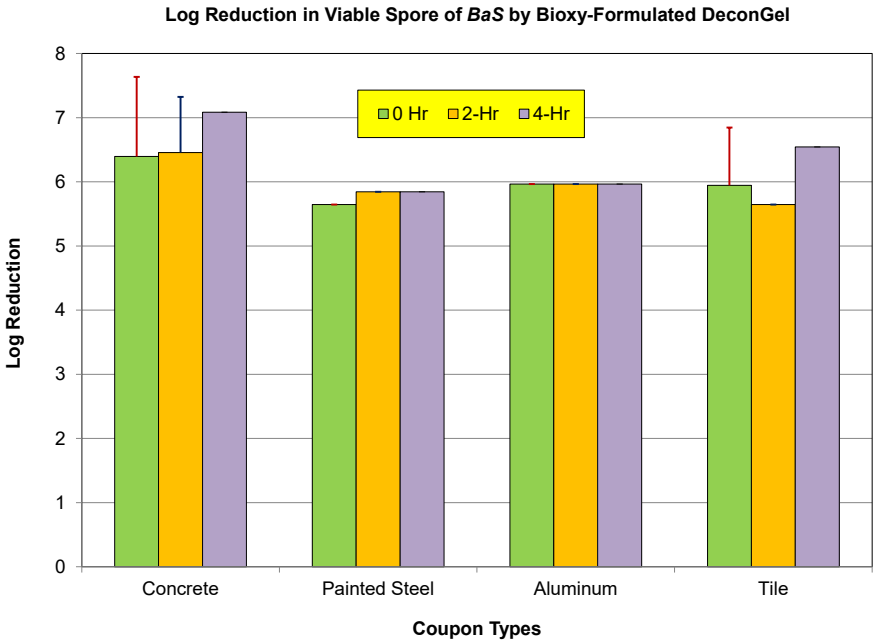

Figure 1. Log Reduction in viable spore of BaS by Bioxy-formulated DeconGel. This formula was tested on concrete, painted steel, aluminium and tile coupons at 0, 2 and 4 hours.

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Author contributions

VKR and FD conceived and designed the experiments. FD, MD and DD formulated "Bioxy Enviro" and supplied the product. VKR, LS and GE performed the experiments. VKR and FD analysed the data. VKR and FD prepared the tables. VKR and FD wrote the main manuscript text. All authors reviewed the manuscript.

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