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Decontamination of Soil Contaminated with *Bacillus anthracis* Ames Spores

Spores of *Bacillus anthracis* (the causative agent for anthrax) are highly resistant to severe environmental conditions and remain viable after decades in soil. Contamination following a wide-area release could overwhelm the nation's remediation capacity, drag cleanup out over many years and result in significant economic impact.

Effective *, widely available, and economical decontamination methods that can be employed on soils in the event of a wide-area release are needed. Table 1 summarizes the results from initial screenings conducted in the laboratory to find efficacious decontaminants for soil. Tests were conducted with 1 cm uncompacted topsoil placed in Petri dishes or glass jars of similar size.

Table 1. Efficacy of Decontaminants on Topsoil Contaminated with *Bacillus anthracis* Ames [3]

Decontaminants Tested (Primary Sporicidal Active Ingredient)	Total Contact Time	Number of Applications During Total Contact Time	Range of Mean Log_{10} Reduction Achieved With Treatment
pH-amended Ultra Clorox® Germicidal Bleach[1] (Hypochlorous acid)	60 min	4	0 to 1
	120 min	8	0 to 1
CASCAD™ SDF (Hypochlorous acid)	60 min	2	0 to 1
	120 min	4	1 to 2
Oxonia Active ® (Peracetic acid)	60 min	6	0 to 1
	120 min	12	1 to 2
Klozur™ (Sodium persulfate) Activated With H_2O_2[2]	24 h	6	1 to 2
	48 h	6	3 to 4

[1] Sodium hypochlorite at 6.15%, sodium hydroxide at <1%, and diluted with sterile filtered water, and pH-amended with acetic acid at 5% to achieve a pH between 6.5 and 7.

[2] Klozur™ is sodium persulfate and activated with hydrogen peroxide· It was tested at relatively long contact times, typical of field-use conditions for remediation of soil contaminated with organic chemicals.

* A minimum log_{10} reduction of 6.0 is generally considered effective decontamination for *B. anthracis.*

DECONTAMINATION OF ARIZONA TEST DUST AND TOPSOIL CONTAMINATED WITH SPORES OF *BACILLUS ANTHRACIS* AMES

Following the screening tests, pH-amended bleach and sodium persulfate/hydrogen peroxide (H_2O_2) were selected for further testing. Additional tests were conducted with pH-amended bleach (at longer contact times) because of its wide availability. Additional tests were conducted with persulfate/H_2O_2 because it showed some moderate effectiveness against anthrax in screening tests. Some other decontaminants shown to be effective against anthrax on building materials were also included in tests for soil.

In order to identify effective decontamination methods on soils, studies using fumigants and liquids on Arizona Test Dust (AZTD) and a garden topsoil (represents a difficult soil to treat in terms of its organic content) were conducted using varying operational parameters.

The fumigants tested were chlorine dioxide (ClO_2) gas; methyl bromide (MeBr); and metam sodium [sodium N-methyldithiocarbamate]. The liquids tested were ClO_2 aqueous solution; pH-amended bleach; and sodium persulfate (Klozur™) activated with hydrogen peroxide (H_2O_2).

Tests were conducted with varying operational parameters that included contact time; number of spray applications (liquids); decontaminant concentration; temperature; soil depth; and soil moisture.

Table 2 summarizes the minimum treatments for each decontaminant that achieved > 6 log_{10} reduction on at least one soil type [1, 3, 4]. Results for gaseous chlorine dioxide are summarized separately in Table 3.

Table 2. Minimum Treatment Required for Effective Decontamination of Soil Inoculated With *Bacillus anthracis* Ames Spores [1, 2]

Decontaminant	Soil Type (1 cm depth)	Minimum Treatment to Achieve > 6 Log_{10} Reduction
pH-amended Ultra Clorox® Germicidal Bleach[1]	Topsoil	Not found [2]
	AZTD	4 applications, 2 h contact time
Aqueous ClO_2 [3]	Topsoil	Not found
	AZTD	Not found
Sodium persulfate activated with H_2O_2 [4]	Topsoil	3 applications every 30 min
	AZTD	3 applications every 60 min

Table 2. Minimum Treatment Required for Effective Decontamination of Soil Inoculated With *Bacillus anthracis* Ames Spores [1,2] *continued*

Decontaminant	Soil Type (1 cm depth)	Minimum Treatment to Achieve > 6 Log_{10} Reduction
Methyl bromide [5]	Topsoil	180 mg/L, 24 h contact time
	AZTD	140 mg/L, 24 h contact time
Metam sodium	Topsoil	160 μL, 7 day contact time, 7 day aeration time, 1mL water added to soil
	AZTD	80 μL, 5 day contact time. no aeration time, no moisture added to soil

[1] One bleach application consisted of 0.5 mL acidified bleach, with mean free available chlorine level of approximately 5,400 ppm and pH 6.5

[2] After 8 spray applications, 7 day contact time < 1 log_{10} reduction was achieved.

[3] Aqueous ClO_2 was tested at most robust condition of 4,000 ppm ClO2, 2 hr contact time, 4 spray applications

[4] One sodium persulfate application consisted of 1 mL 0.5 M sodium persulfate followed by 1mL 8% H_2O_2; all tests used 7 day contact time.

[5] All methyl bromide tests conducted at 25 °C and RH uncontrolled (all but one test had > 75% RH).

Table 3 summarizes the effect soil depth has on the efficacy of ClO_2 gas and the contact time needed to achieve at least a 6 log_{10} reduction under two different RH conditions. Relative humidity was varied based on observations made in earlier work that RH can affect the efficacy of chlorine dioxide gas (RH was not an experimental variable in the other studies) .

Table 3. Minimum Contact Time Required to Achieve > 6 Log_{10} Reduction Using Chlorine Dioxide Gas [1] on Soil Contaminated With *Bacillus anthracis* Ames Spores [4]

Soil Type	Percent Relative Humidity	Soil Depth (cm)	Minimum Contact Time (h) to Achieve > 6 Log_{10} Reduction
Topsoil	75	1	2
AZTD	75	1	2
Topsoil	85	1	2
AZTD	85	1	2
Topsoil	75	2	Not found after 4 hours
AZTD	75	2	2
Topsoil	85	2	Not found after 4 hours
AZTD	85	2	2

[1] Concentration 3,000 ppm by volume

DESCRIPTION OF DECONTAMINANTS TESTED

Fumigants

- **Chlorine Dioxide Gas [4]**

  Chlorine dioxide has been shown to be an effective decontaminant on a variety of porous and non-porous indoor materials contaminated with *B. anthracis* spores.

- **Methyl Bromide [2]**

  Methyl bromide has been shown to be an effective decontaminant on a variety of porous and non-porous indoor materials contaminated with *B. anthracis* spores. It is also used as a soil and agricultural commodity fumigant, but is being phased out under the Montreal Protocol for Substances that Deplete the Ozone Layer.

- **Metam Sodium (Sodium N-methyldithiocarbamate) [2]**

  (EPA Reg. No. 11448-107) Metam sodium is the most widely used soil fumigant in the U.S.

Liquids

- **CASCAD™ SDF (Hypochlorous acid) [3]**

  CASCAD is produced by mixing together two solutions that form hypochlorous acid, hydrogen peroxide, along with foaming and buffering agents.

- **Oxonia Active ® (Peracetic acid) [3]**

  Contains 5.8% peracetic acid. Registered with EPA as a sterilant (1677-129).

- **Aqueous Chlorine Dioxide [1]**

  ClO_2 was produced using water, hydrochloric acid, sodium hypochlorite, and sodium chlorite. The concentration of ClO_2 was adjusted by the amount of sodium hypochlorite.

- **pH-amended Bleach (acidified) [2, 3]**
  Ultra Clorox ® Germicidal Bleach (EPA Reg. No. 67619-8) was used to make pH-amended bleach, which is not registered with EPA. The amended bleach was made by mixing 9.4 parts water, 1 part Ultra Clorox® Germicidal Bleach, and 1 part 5% acetic acid (for example, vinegar). The resulting solution should have a pH of about 6.8 and a mean total chlorine content of about 6,200 ppm.

- **Sodium persulfate (Klozur™) Activated with Hydrogen Peroxide [2, 3]**

  In the initial screenings of soil decontaminants, Klozur ™ was shown to be moderately effective against *B. anthracis* Ames on soil (see Table 1). It is typically used to remediate soil contaminated with organic chemicals, and was tested against *B. anthracis* spores at the conditions used for organic chemical remediation.

LESSONS LEARNED FROM SOIL DECONTAMINATION INVESTIGATIONS

Minimum treatment conditions to achieve a 6 $\log_{10}$ or greater reduction for *B. anthracis* Ames spores in both tested soil types were found for sodium persulfate activated with hydrogen peroxide, methyl bromide, and metam sodium, but were not found for pH-amended bleach or aqueous ClO_2.

The Arizona Test Dust was generally easier to decontaminate then the proprietary garden topsoil, but this depends on the decontaminant.

Tests using ClO_2 gas showed decontaminant efficacy decreased when soil depth increased.

CONTACT INFORMATION

For more information, visit the EPA Web site (http://www2.epa.gov/homeland-security-research).

If you have difficulty accessing this PDF document, please contact Kathy Nickel (Nickel.Kathy@epa.gov) or Amelia McCall (McCall.Amelia@epa.gov) for assistance.

Technical Contact: Joseph Wood (wood.joe@epa.gov)

General Feedback/Questions: Kathy Nickel (nickel.kathy@epa.gov)

REFERENCES

[1] U.S. EPA. 2012. *Decontamination of Indoor and Outdoor Materials with Aqueous Chlorine Dioxide Solutions.* Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-12/516.

[2] U.S. EPA. 2013. *Decontamination of Soil Contaminated with Bacillus anthracis Spores-report*. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-13/110.

[3] U.S. EPA. 2010. *Evaluation of Liquid and Foam Technologies for the Inactivation of Bacillus anthracis Spores in Topsoil.* Investigation Report. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-10/080.

[4] U.S. EPA. 2012. *Inactivation of Bacillus anthracis Spores in Soil Matrices with Chlorine Dioxide Gas.* Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-12/517.

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