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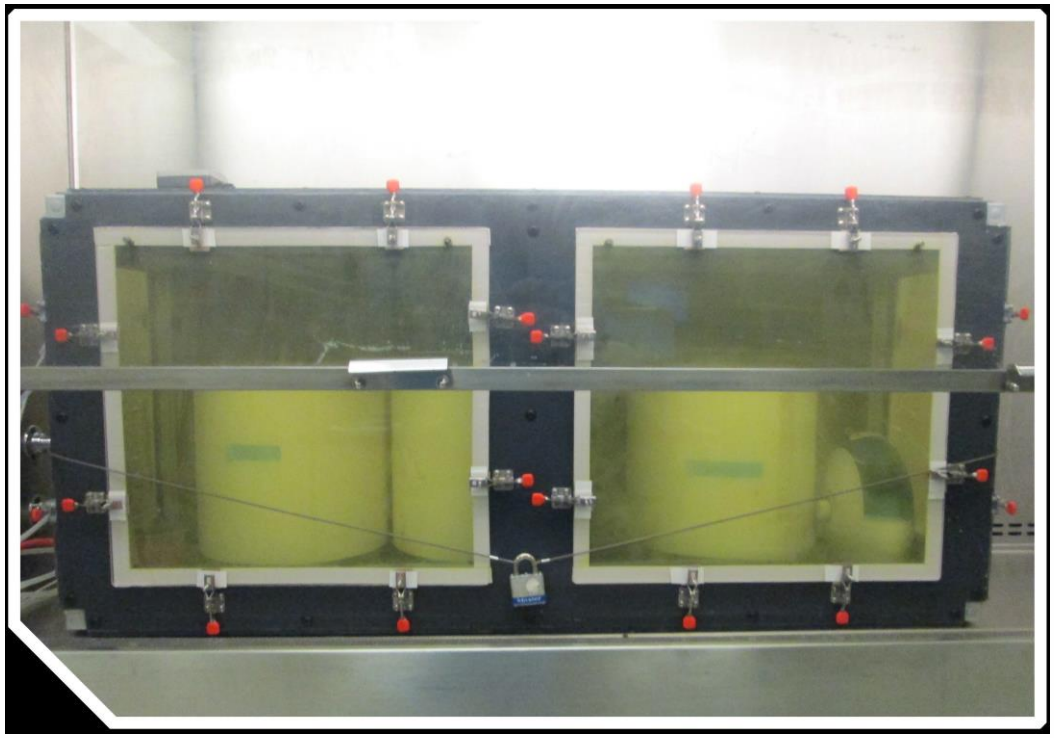
Update on Research for Decontaminating Soil Contaminated with *Bacillus anthracis* Spores



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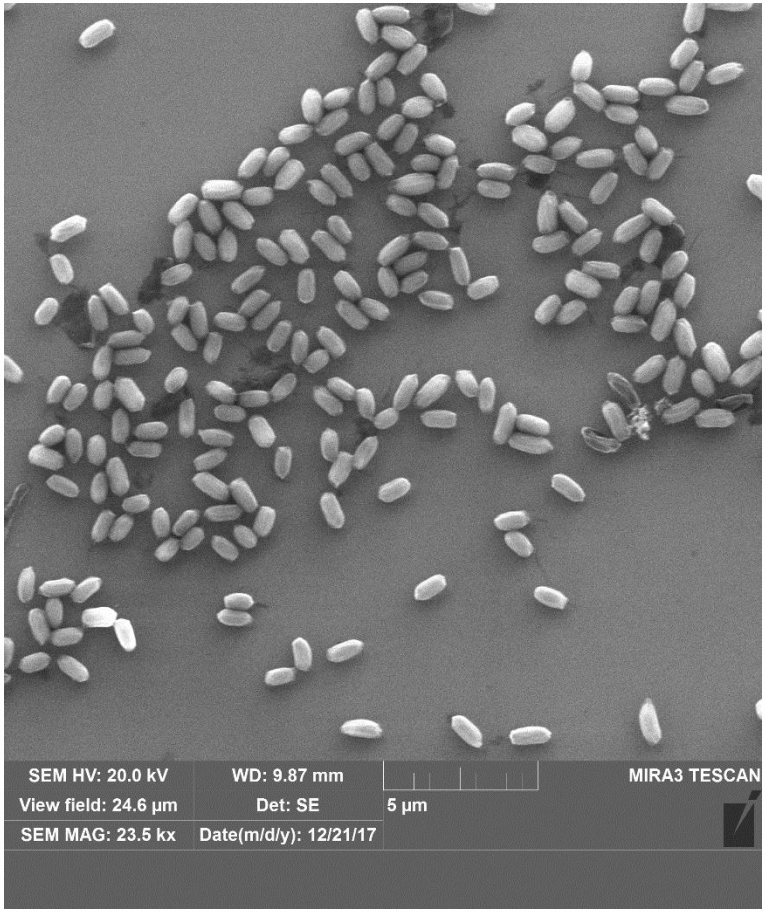
Acknowledgements and Disclaimer

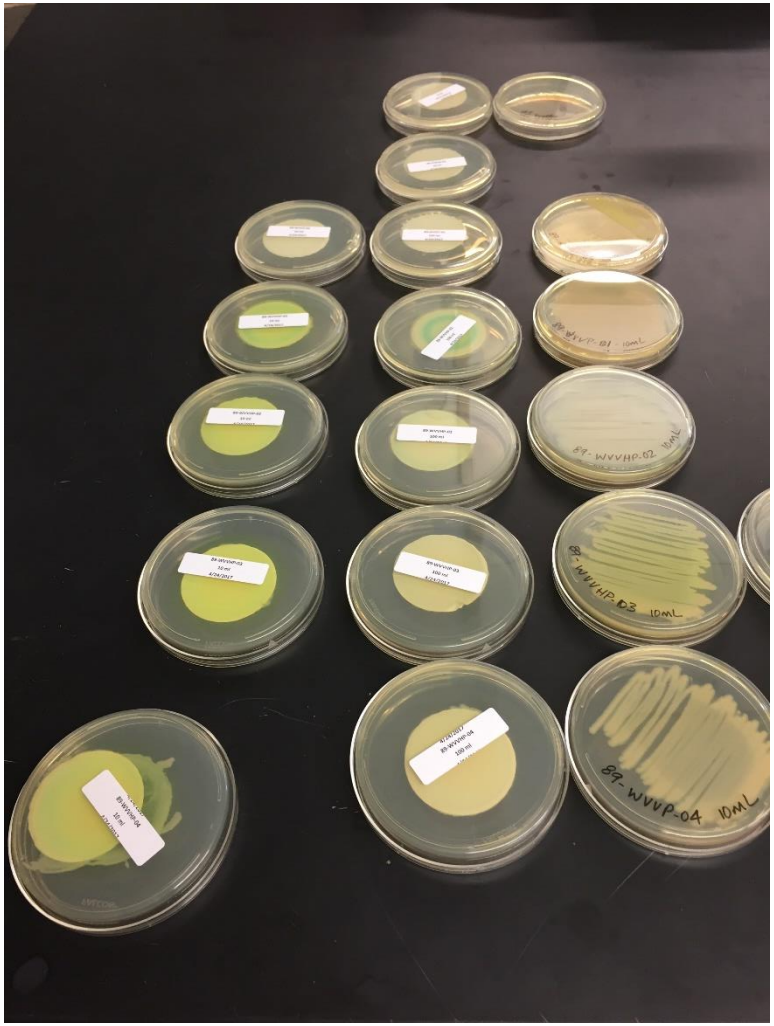
- EPA advisory team:
  - Leroy Mickelsen, Shannon Serre, Worth Calfee, Lukas Oudejans, Erin Silvestri
- Performer: MRI Global



B. atrophaeus spores in liquid suspension inoculated on to silicon wafer. SEM photo credit: Anne Mikelonis, Katherine Ratliff, Jason Weinstein

Outline of Presentation

- Problem definition, purpose of study
- Previous soil decontamination results and findings
- Overview of present study
- Methods and results of present soil decon study
- Summary



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Problem Definition

- Wide area release of *B. anthracis* spores would disperse and contaminate outdoor environment, including soils
- Bacterial spores may survive/persist in soils for decades
- Inactivation of spores in soil is difficult due to soil porosity, organic content, depth of spores in soil

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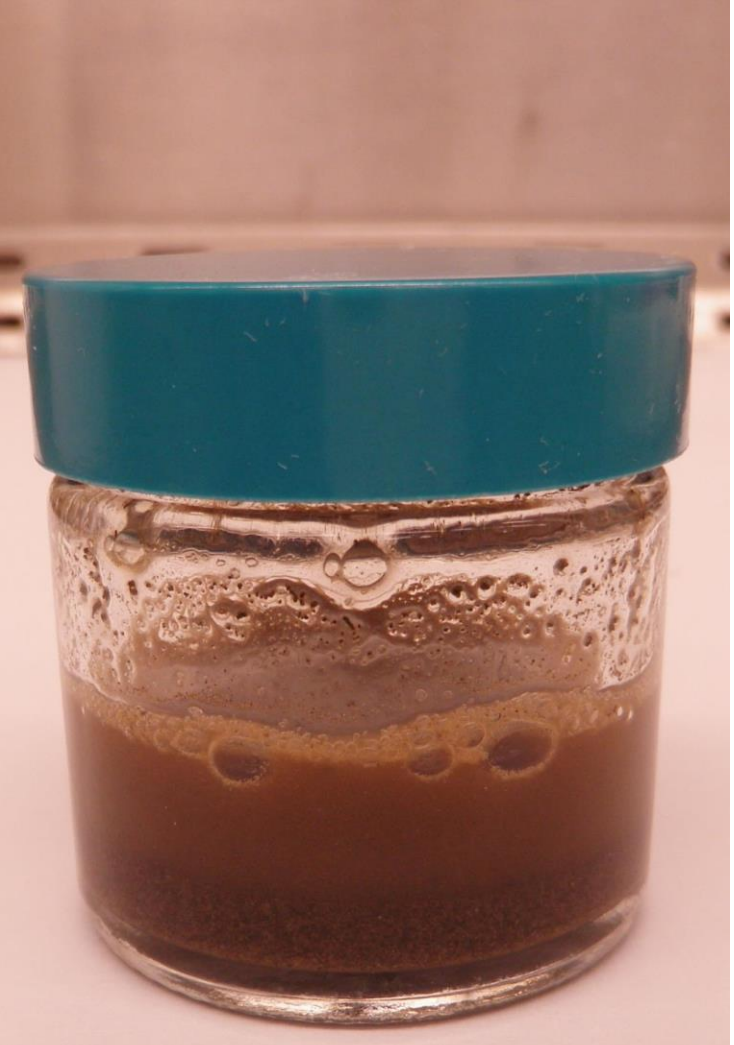
Purpose, Objectives, and Rationale of the Research

- Evaluate decontamination efficacy as a function of following parameters:
  - Decontaminant
  - Type of soil
  - Soil depth
  - Decon parameters

Previous Soil Decon Technology Evaluations

- Tests used small amounts of soil (1-2 cm depth) in Petri dishes or small jars, using topsoil or Arizona Test Dust
- Chemical technologies that provided > 6 log reduction (LR) in at least one test condition:
  - Methyl bromide (MeBr)
  - Chlorine dioxide (ClO_2) gas
  - Metam sodium
  - Sodium persulfate activated with aqueous hydrogen peroxide

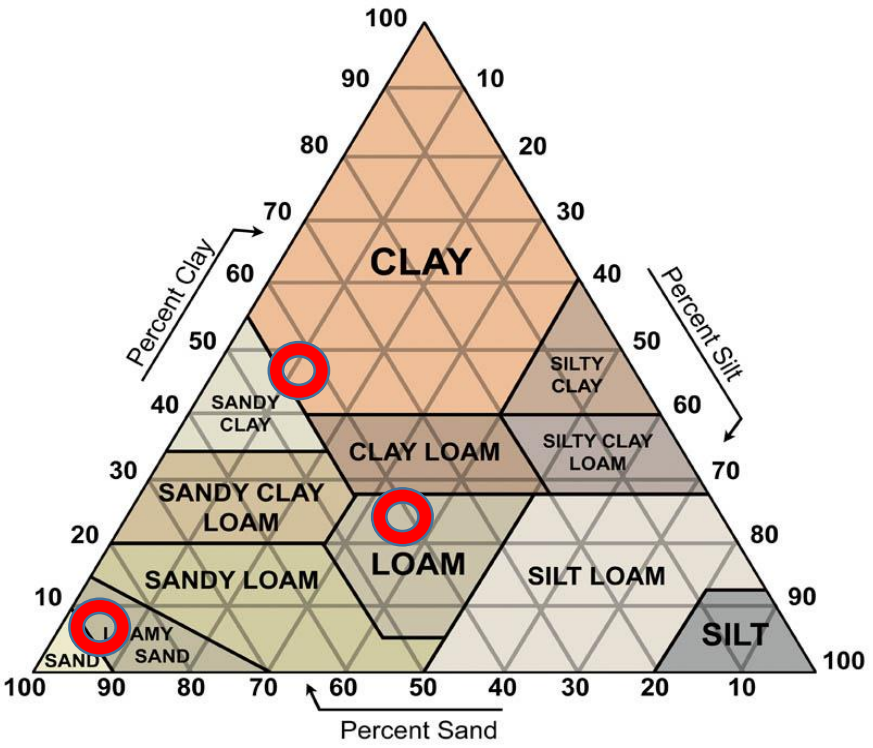


Previous Soil Decon Technology Evaluations

- What decontaminants were not effective?
  - pH adjusted bleach
  - Dichlor with surfactant (CASCAD)
  - Peracetic acid
  - Aqueous ClO_2

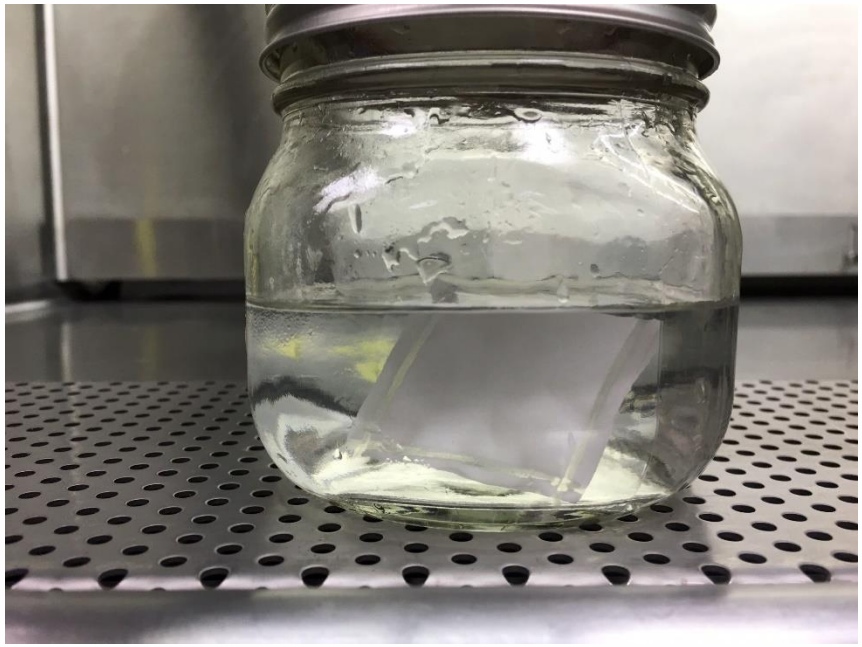
Present Study Overview

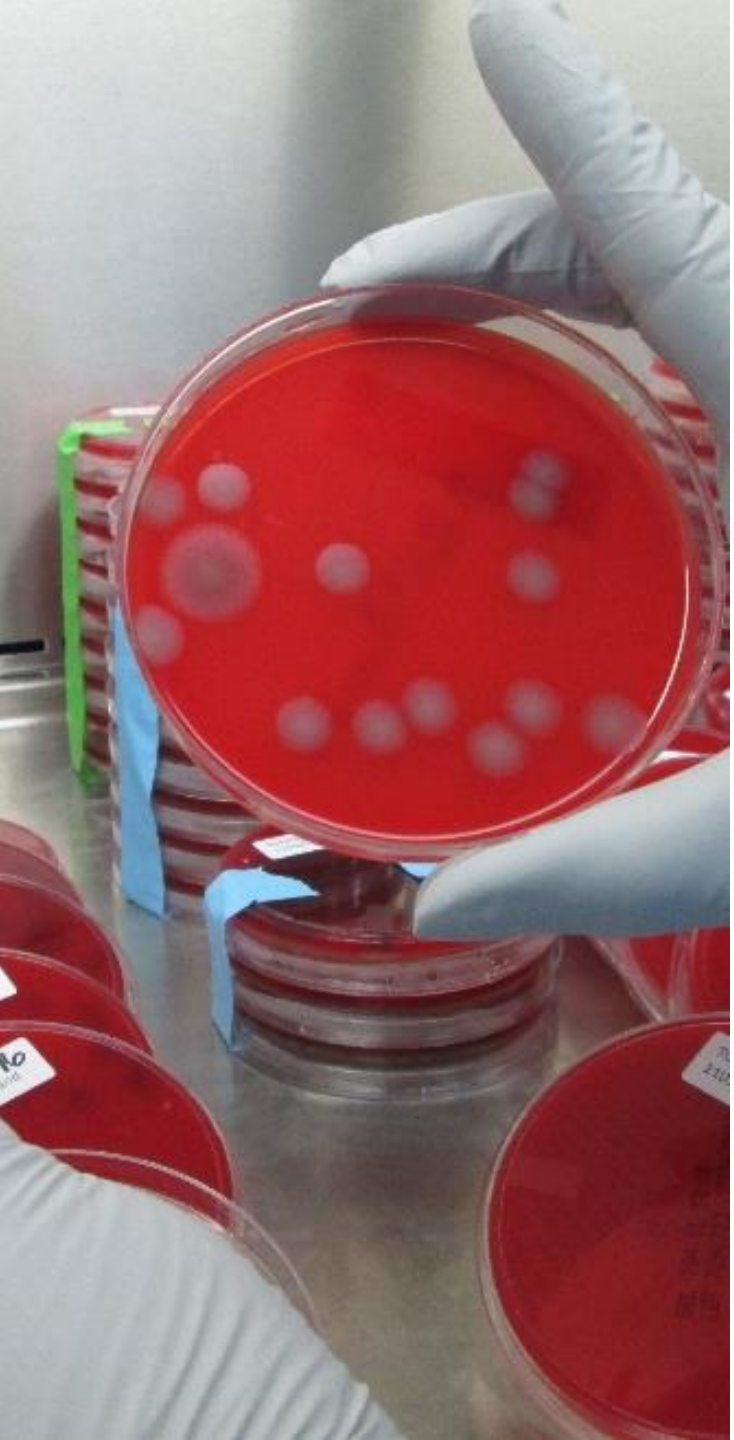
- Larger scale than previous tests:
  - 10 inch diameter, 12 inch deep soil columns
  - spores placed center of column, increments of 1 inch or ½ inch; from top of soil column down to 5 inch depth
- Use of a topsoil, sandy soil and clay soil
- Soil density and moisture measured prior to each test
- Chemical analyses of soils conducted at beginning of project



Present Study Overview

- Focus on decon techs demonstrated to be effective in previous tests:
  - ClO_2 gas
  - activated sodium persulfate
  - methyl bromide
- Spores of *B. anthracis* Ames inoculated (liquid) in custom-made carrier soil packets (CSP)
  - contained sterilized soil and wrapped in Tyvek (ClO_2 and MeBr) or PVDF (for persulfate tests) pouches
- CSPs placed at 0-5 inch depth





Microbiological Methods

- *B. anthracis* (Ames) was used in tests
  - Used PCR analysis to verify that stock contained pX01 and pX02 virulence plasmids
- 10^9 CFU/mL; 0.1 mL inoculated on each CSP
- Dilution plating, colony counting on sheep blood agar in triplicate; incubated 16-20 hr 35 ° C
- Spores extracted with 10 mL sterile phosphate buffered saline (PBS) with 0.1% Triton-X 100; samples agitated 15 minutes at 200 rpm.

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Study Test Matrix Overview

Decontaminant	No. Tests	Decontaminant Concentration	Exposure Time (hrs)	Avg. RH %
Chlorine Dioxide Gas	7	8.7 - 14.6 mg/L (~3000-5000 ppm)	3 - 28	74 – 86
Activated Sodium Persulfate	3	0.5 – 1.0 M 50/50 w/ H_2O_2 0.09 – 0.18 mL/g 1 – 6 doses	144 - 168 (6 - 7 days)	91 – 93
Methyl Bromide	4	224 – 325 mg/L	48 – 66	76 – 79

All tests conducted at ambient temps, i.e., 20-25 ° C

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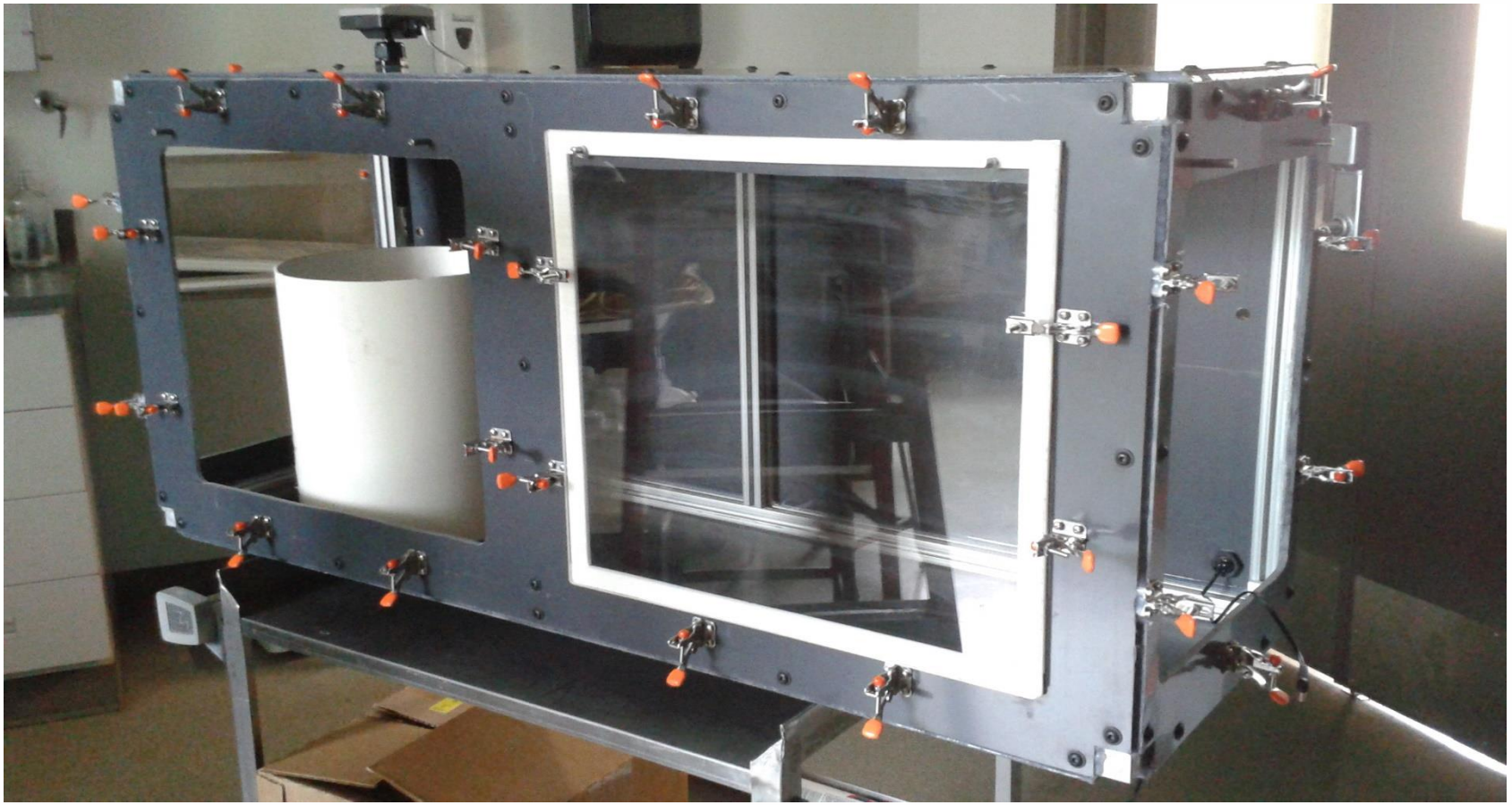
Test Chamber



ClO_2 Decontamination Test Methods

- ClO_2 produced, controlled, and measured using a ClorDiSys Minidox system, which also provided humidification

- Temperature and RH were monitored with a HMD40/50 probe from Vaisala

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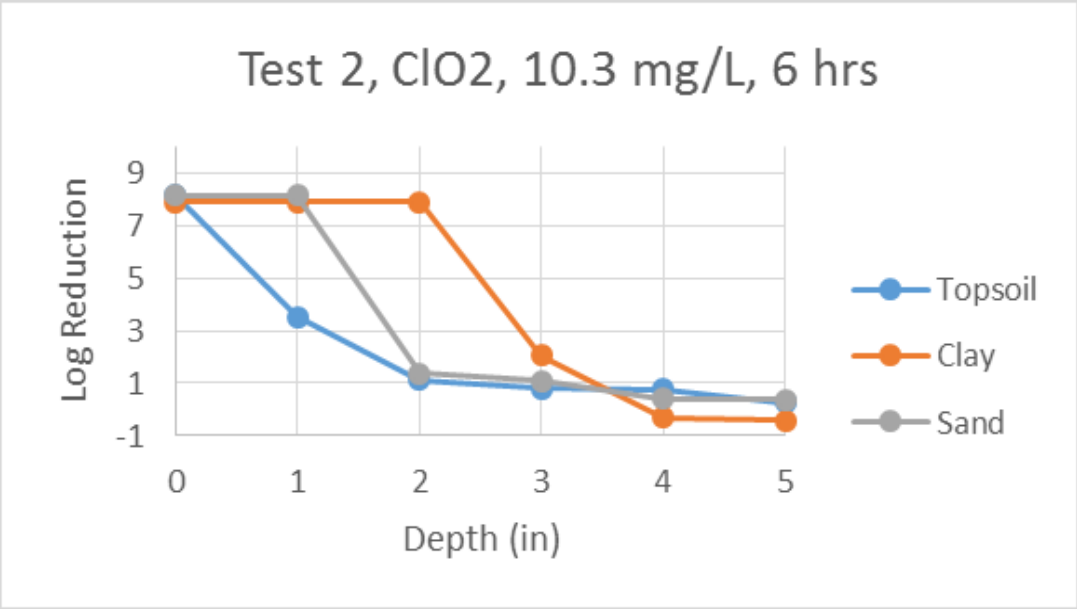
Chlorine dioxide gas methods/results

Test No.	Depth(s) Tested (inches)	Avg. ClO_2 Conc. (mg/L)	Avg. RH (%)	Contact Time (hrs)	Other Conditions	Max depth (inches) achieving ≥ 6 LR topsoil	Max depth (inches) achieving ≥ 6 LR clay	Max depth (inches) achieving ≥ 6 LR sandy
1	0, 1, 2, 3, 4, 5	8.7	76.7	3		0	0	0
2	0, 1, 2, 3, 4, 5	10.3	79.6	6		0	2	1
3	0, 0.5, 1, 1.5, 2, 2.5	8.9	73.5	3	Saturated soil	0	2.5	2
4	0, 1, 2, 3, 4, 5	10.1	80.6	24		1	5	5
5	0, 1, 2, 3, 4, 5	9.3	80.1	24	Saturated soil	0	5	2
6	0, 1, 2, 3, 4, 5	14.6	82.5	27.5		1	5	5
7	0, 1, 2, 3, 4, 5	9.4	85.8	7.75	Compacted soil	0	3	2

ClO_2 Decon Efficacy Results Summary

- All spores left on top of soil column were completely inactivated in every test, for every soil type
- **Topsoil:** Found effective down to 1 inch in two tests.
- **Sand:** Full decontamination down to 2" for most of the test conditions. Furthermore, two of the three 24-hr tests showed complete kill (≥7 LR) at all depths.
- **Clay** had full decontamination down to 3" for most of the test conditions. Two of the three 24-hr tests showed complete kill (≥7 LR) at all depths.



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Activated Sodium Persulfate Methods

- Used either 0.5 or 1.0 molar sodium persulfate activated with 8% hydrogen peroxide solution
- 1-6 doses of solution, depending on material
- Contact times of 6-7 days
- Extremely reactive with topsoil; produced a vigorous foaming reaction upon application

Activated Sodium Persulfate Results Summary

Overall: All spores left on top of soil column were completely inactivated in every test, for every soil type – with just one exception

Topsoil: Effective to 0.5" for the 0.5M solutions and to 1" for the 1.0 M solution, using 6 doses.

Clay: Complete decontamination down to 5" with only two applications of activated sodium persulfate.

Sandy soil: less reactive; formed a slurry Decontamination was effective to 1" depth with 6 doses.

Methyl Bromide Decon Test Methods

- MeBr concentrations ranged from 224 to 325 mg/L (56,000 to 81,250 ppm)
- Exposure times 48 to 65 hours
- RH levels ≥ 75%
- Initial conditions selected based on previous lab tests showing effective decontamination
- Poor efficacy prompted us to confirm again via PCR that the microorganism we were working with was in fact *B. anthracis*

Methyl Bromide Results Summary

- Unable to achieve > 6 LR down to 1 inch in any test, for any soil type
- Efficacy was observed to be somewhat higher for topsoil and sand, and lowest for the clay soil
- Efficacy was similar across all depths for a particular soil and test condition
  - suggests penetration of MeBr gas through soil matrix not a limiting factor
- Uncertain why MeBr results inconsistent with previous EPA decontamination studies using MeBr.
  - Further research may be needed to clarify this discrepancy.

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Summary

- Even after several studies, soil decontamination remains particularly challenging
- Topsoil generally most difficult to decontaminate, and clay material relatively easier, for oxidative technologies (ClO_2, persulfate)
  - Topsoil required excessive decon conditions to be effective to just one inch depth
- Contradictory, unexpected, unsuccessful results for methyl bromide

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