EPA 600/R-14/226 | August 2014 | www.epa.gov/research

United States
Environmental Protection
Agency

Interactions of ClO_2 and H_2O_2 Fumigants with Dirt and Grime on Subway Concrete

ASSESSMENT AND EVALUATION REPORT

Office of Research and Development
National Homeland Security Research Center

August 2014
EPA/600/R-14/226

Interactions of ClO_2 and H_2O_2 Fumigants with Dirt and Grime on Subway Concrete

Assessment and Evaluation Report

National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

The United States Environmental Protection Agency, through its Office of Research and Development's National Homeland Security Research Center, funded and managed this investigation under EPA Contract Number EP-C-09-027, Work Assignment 4-12 with ARCADIS U.S., Inc. This report has been peer and administratively reviewed and has been approved for publication as an Environmental Protection Agency document. It does not necessarily reflect the views of the Environmental Protection Agency. No official endorsement should be inferred. This report includes photographs of commercially available products. The photographs are included for purposes of illustration only and are not intended to imply that EPA approves or endorses the product or its manufacturer. Environmental Protection Agency does not endorse the purchase or sale of any commercial products or services.

Questions concerning this document or its application should be addressed to:

Lukas Oudejans, Ph.D.
Decontamination and Consequence Management Division
National Homeland Security Research Center
U.S. Environmental Protection Agency (MD-E343-06)
Office of Research and Development
109 T.W. Alexander Drive
Research Triangle Park, NC 27711
Phone: 919-541-2973
Fax: 919-541-0496
E-mail: Oudejans.Lukas@epa.gov

Acknowledgments

The following individuals and organizations are acknowledged for review of this document:

United States Environmental Protection Agency:

Office of Solid Waste and Emergency Response, Office of Emergency Management

Larry Kaelin
Shannon Serre (on detail from ORD-NHSRC)

Office of Research and Development, National Homeland Security Research Center

Joe Wood
Ramona Sherman (QA review)

The support and efforts provided by ARCADIS U.S., Inc., are gratefully acknowledged.

Table of Contents

List of Figures

List of Tables

List of Acronyms and Abbreviations

ADA	aerosol deposition apparatus
APPCD	Air Pollution Prevention and Control Division
ATCC	American Type Culture Collection
ATI	Analytical Technologies, Inc.
B.	*Bacillus*
CBR	chemical, biological, or radiological
CBRN	Chemical, Biological, Radiological, and Nuclear
CFU	Colony Forming Unit(s)
ClO_2	chlorine dioxide
CMAD	Consequence Management Advisory Division
COC	chain of custody
CT	concentration × time
DAS	data acquisition system
DHS	Department of Homeland Security
DQO	Data Quality Objective
EMS	Environmental Monitoring System
EPA	U. S. Environmental Protection Agency
EtO	ethylene oxide
H_2O_2	hydrogen peroxide
HSPD	Homeland Security Presidential Directive
HSRP	Homeland Security Research Program
ID	identification
LR	log reduction
MDI	metered dose inhaler
MIT	Massachusetts Institute of Technology
mSM	modified Standard Method
MTA	Metropolitan Transportation Authority
NA	not applicable
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OSWER	Office of Solid Waste and Emergency Response
pAB	pH-adjusted bleach
PBST	Phosphate Buffered Saline with 0.05 % TWEEN® 20
ppm	part(s) per million
ppmv	part(s) per million volume
PRB	Polyester-Rayon Blend

PTFE	Polytetrafluoroethylene
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RH	relative humidity
RSD	relative standard deviation
RTP	Research Triangle Park
SD	standard deviation
SOP	standard operating procedure
VHP®	(STERIS-registered) Vaporized Hydrogen Peroxide
VPHP	Vapor-phase Hydrogen Peroxide

Executive Summary

This project supports the mission of the U.S. Environmental Protection Agency's (EPA) Office of Research and Development's (ORD) Homeland Security Research Program (HSRP) to conduct research and develop scientific products that improve the capability of EPA to carry out its homeland security responsibilities. Improving the capability for transit systems to rapidly recover from a biological event has been identified as a high priority need. The remediation of a transportation hub, like a subway system, may require the use of volumetric decontamination approaches, such as fumigation with chlorine dioxide (ClO_2) or hydrogen peroxide (H_2O_2).While previous National Homeland Security Research Center (NHSRC) studies have shown these fumigants to be highly efficacious if applied under specific environmental conditions (temperature and relative humidity (RH)), it is unclear what the impact of dirt and grime is on the efficacy of these fumigants on realistic (subway) building materials. The presence of dirt and grime may result in a change in sporicidal activity of the fumigant and may therefore require changes in operational fumigation conditions to reach remediation goals. The primary objective of this research was to evaluate the impact that dirt and grime, as present on unpainted subway concrete, may have on the fumigation efficacy.

The impact of dirt and grime on concrete surfaces was also investigated as part of a material demand study. Building materials like concrete may impact the fumigant concentration by either sorption or decomposition of the decontaminant. Consequently, higher input fumigant concentrations would be required to achieve and maintain the targeted effective concentration within an enclosed interior space. Previous NHSRC material demand efforts for ClO_2 and vaporized hydrogen peroxide (VHP®) used clean (concrete) surfaces. Dirt and grime may increase material demand when present on a material associated with low or no demand while material demand may possibly decrease if dirt and grime forms a protective layer on a material associated with higher demand.

Other objectives include determining which sampling procedure provides better recovery from grimed and cleaned concrete using a prescribed method from the New York City Metropolitan Transportation Authority (MTA). A method was developed using metered dose inhalers (MDIs) to inoculate 1.5" coupons of subway concrete. Three surface sampling methods (sponge wipe, cloth wipe, and vacuum sock) were tested. All three methods showed a recovery comparable to the recovery from stainless steel coupons, but the sponge wipe method had higher and more repeatable recovery.

Fumigation results for subway concrete using ClO_2 were found to be in agreement with fumigation data available for unpainted cinder block. Here, greater than 6-log reductions in *Bacillus* spores were observed for 1500 ppmv ClO_2, 75% RH and ≥ 4 hours (h) contact time or 500 ppmv ClO_2, 75% RH and ≥ 6 h contact time (shortest contact time tested). Though this investigation suggests that fumigation of washed subway concrete can result in different efficacy values than fumigation of the unwashed subway concrete, the differences are statistically not significant and do not suggest that the presence of grime on concrete would affect fumigation efficacy.

Fumigation results for subway concrete using VPHP cannot be immediately compared to clean concrete data as available VPHP fumigation data for concrete are limited to painted concrete. A greater than 6-log reduction in *Bacillus* spores on subway concrete was observed for 250 ppm H_2O_2 (as generated using Steris VHP® technology), 20% RH and a ≥ 4 h contact time. The 6-log reduction in spores was not reached at the longest contact time (10 h) at the 150 ppmv H_2O_2 concentration. Observed differences in

log reduction between washed and unwashed subway concrete following VHP® fumigation were not statistically significant.

The grimed subway concrete had no detectable material demand when using the ClO_2 fumigant. This is consistent with previously obtained data for clean unpainted concrete cinder block. Material demand studies using the subway concrete were not conducted for VHP® based on limited availability of subway concrete material. In addition, previous VHP® material demand tests showed that the presence of concrete cinder block coupons had a large impact on maintaining the VHP® concentration due to decomposition of VHP®. In the presence of concrete cinder block, a high (2x) increase in generator output was required to maintain the target concentration due to degradation of the hydrogen peroxide at the surface.

Reported results presented here were obtained from a small scale study with a limited amount of subway concrete (surface) available. Extrapolation of these results to a full scale subway station fumigation process should be made with caution. Additional fumigation testing on an intermediate or large scale would assist in such extrapolation of results. Levels and composition of dirt and grime may also vary significantly throughout a subway (tunnel) system.

1 Introduction

This project supports the mission of the U.S. Environmental Protection Agency's (EPA) Homeland Security Research Program (HSRP) by providing relevant information pertinent to the decontamination of contaminated areas resulting from an act of terrorism. Under Homeland Security Presidential Directive (HSPD)-10, the U.S. Department of Homeland Security (DHS) is tasked to coordinate with other appropriate Federal departments and agencies to develop comprehensive plans that "provide for seamless, coordinated Federal, state, local, and international responses to a biological attack." As part of these plans, EPA, in a coordinated effort with DHS, is responsible for "developing strategies, guidelines, and plans for decontamination of persons, equipment, and facilities" to mitigate the risks of contamination following a biological weapons attack.

EPA's National Homeland Security Research Center (NHSRC) provides expertise and products under the HSRP that can be used widely to prepare for, respond to, and recover from public health and environmental emergencies arising from terrorist threats and incidents. The HSRP's research on biological agent decontamination supports EPA's Office of Solid Waste and Emergency Response (OSWER) and the Office of Pesticide Programs (OPP). OSWER and its Special Teams, which include the Chemical, Biological, Radiological, and Nuclear (CBRN) Consequence Management Advisory Division (CMAD), support the emergency response functions carried out by the Regional Offices. The OPP supports the decontamination effort by providing expertise on (biological) agent inactivation and ensuring that the use of pesticides in such efforts is done in accordance with applicable laws. Close collaboration between the different program offices having homeland security responsibilities is sought to rapidly increase EPA's capabilities to help the Nation recover from a terrorist event involving the intentional release of chemical, biological, or radiological (CBR) materials.

In 2001, the introduction of a few letters containing *Bacillus anthracis* spores into the U.S. Postal Service system resulted in the contamination of several facilities. In the event of a biological incident in a transportation hub like a subway system, remediation may require the use of various remediation options including volumetric decontamination approaches such as fumigation as an effective decontamination method. Rapid decontamination of subways and other transportation infrastructure is not only critical for the reoccupancy of the contaminated area but also for the surrounding areas that use the transit system.

Previous NHSRC studies have shown that fumigants like chlorine dioxide (ClO_2) and hydrogen peroxide (H_2O_2) vapors can be highly efficacious if applied under specific application (temperature and relative humidity (RH)) conditions. It is, however, unclear what the impact is of dirt and grime on realistic building materials on the fumigation efficacy. Such presence may result in change in sporicidal activity of the fumigant and may also require changes in operational fumigation conditions to reach remediation goals due to changes in material demand.

1.1 Project Objectives

The primary objective was to evaluate the impact that dirt and grime, as present on unpainted subway concrete, has on ClO_2 and VPHP fumigation efficacy. Secondary objectives were (1) to identify which sampling procedure provided a better recovery of *B. anthracis* (surrogate) spores from grimed and cleaned concrete, and (2) to measure any gross material demand presented by the presence of dirt and grime on concrete.

To meet the project objectives, this study was comprised of the following four tasks:

1. Modification of the aerosol deposition method. An aerosol deposition method described by Lee et al[1]. and Calfee et al. [2] was designed for inoculation of a 12" x 12" square. This aerosol deposition method was modified to deposit on 1.5" x 1.5" concrete coupons.

2. Determination of a surface sampling method using the modified aerosol deposition procedure. Coupons were sampled using three techniques (sponge wipe, wetted wipe, and 37-mm vacuum filter) to determine one suitable sampling method for use in decontamination tests.

3. Decontamination tests of subway concrete coupons by fumigation followed by sampling to determine fumigation efficacy.

4. Material demand tests for ClO_2 fumigant (only).

1.2 General Approach

The general process investigated in this project was the decontamination of unpainted concrete surfaces contaminated with *Bacillus* spores (i.e., surrogates of *B. anthracis*). Decontamination can be defined as the process of inactivating or reducing a contaminant in or on humans, animals, plants, food, water, soil, air, areas, or items through physical, chemical, or other methods to meet a cleanup goal.

For this effort, decontamination methods included fumigation with ClO_2 and Vapor-phase Hydrogen Peroxide (VPHP). Concrete from the floor of the New York Metropolitan Transit Authority (MTA) Old South Ferry Station was made available for this research. Sections (coupons) of this subway concrete were inoculated via aerosol deposition. Some coupons underwent fumigation, and recovery of *Bacillus* spores from fumigated coupons was compared to recovery from coupons that were inoculated but not fumigated (positive control coupons). Quality Control (QC) samples such as procedural blank coupons (coupons that undergo the fumigation process but that are not inoculated) and negative controls (coupons that do not undergo the fumigation process and are not inoculated) were also included to monitor for cross-contamination. All samples were analyzed for the quantitative determination of viable spores.

Each of the described tests were conducted in accordance with internal miscellaneous operating procedures (MOPs), to ensure repeatability and adherence to the data quality validation criteria set for this project.

1.2.1 Definitions of Effectiveness

Quantification of Colony Forming Unit (CFU) counts per coupon occurred as a calculated product of the average counted number of CFU and extraction volume (mL) and divided by the product of plated volume (mL) and tube dilution factor. Efficacy is defined as the extent (by log reduction) to which the agent extracted from the coupons after the treatment with the decontamination procedure is reduced below the agent extracted from positive control areas (not exposed to the decontamination procedure). Efficacy (as the log reduction [LR]) was calculated using Equation 1-1 for each material within each combination of decontamination procedure (i) and test material (j) as:

$$LR_{ij} = \sum_{c=1} \log(CFU_{ijc}) / N_{ijc} - \sum_{k=1} \log(CFU_{ijk}) / N_{ijk} \qquad (1\text{-}1)$$

where:

LR_i = the average log reduction of spores on a specific material surface

$\sum_{c=1} \log(CFU_{cj}) / N_C$ = the average of the logarithm of the number of viable spores (determined by CFU) recovered on the control coupons [*c* = control, *j* = coupon number, and N_C is the number of coupons (1, *j*)]

$\sum_{k} \log(CFU_S) / N_t$ = the average of the logarithm of the number of viable spores (determined by CFU) recovered from the surface of a decontaminated coupon [*S*= decontaminated coupon, *k* = coupon number, and N_t is the number of coupons tested (1, *k*)]

When no viable spores were detected, the detection limit of the sample was used, and the efficacy reported as greater than or equal to the value calculated by Eqn. 1-1. The detection limit of a sample depends on the analysis method and so may vary. The detection limit of a plate is assigned a value of 1 CFU, but the fraction of the sample plated varies. For instance, the detection limit of a 0.1 mL plating of a 20 mL sample suspension is 200 CFU (1 CFU/0.1 mL * 20 mL), but if all 20 mL of the sample were filter plated, the detection limit would be 1 CFU.

The standard deviation of $\mathrm{LR_i}$ is calculated by Eqn. 1-2:

$$SD_{LR_{ij}} = \sqrt{\frac{\sum_{k=1}^{N_s} \left(x_{ijk} - LR_{ij}\right)^2}{N_{ijk} - 1}} \qquad (1\text{-}2)$$

where:

SD_{LR_i} = Standard deviation of $\mathrm{LR_i}$

LR_{ij} = the average log reduction of spores on a specific material surface

x_{ijk} = The average of the log reduction of each from the surface of a decontaminated coupon (Equation 1-3)

$$x_{ijk} = \frac{\sum_{k} \{\sum_{C=1} \log(CFU_{ijc}) / N_C - \log(CFU_{ijk}))}{N_{ijk}} \qquad (1\text{-}3)$$

where:

$\sum_{c} \log(CFU_{ijc}) / N_{ijc}$ = Represents the "mean of the logs", the average of the logarithm transformed number of viable spores (determined by CFU) recovered on the control coupons [*C*= control, *j* = coupon number, and N_C is the number of coupons (1, *j*)]

CFU_{ijk} = Number of CFU on the surface of the k^{th} decontaminated coupon

2 Materials and Methods

2.1 Fumigation Chamber

An opaque chamber (Plas-Labs 830 series glove box, Plas-Labs, Inc., Lansing, MI, USA) maintained and controlled a leak-free fumigation atmosphere and allowed for the periodic addition and removal of coupons during fumigation. Chlorine dioxide was provided by a ClorDiSys "GMP" ClO_2 generator (ClorDiSys Solutions, Inc., Lebanon, NJ, USA). The generator includes real-time feedback control of concentration via an internal Environmental Monitoring System (EMS) photometric monitor. VPHP was provided by a STERIS Vaporized Hydrogen Peroxide (VHP)® 1000 ED (STERIS Corp., Mentor, OH, USA). The VHP® 1000 ED was connected through a custom-designed control system using a feedback loop from a data acquisition system (DAS).

Humidity of the chamber was controlled by the DAS. A model HMD53 Vaisala RH/temperature sensor (Vaisala, Inc., Helsinki, Finland) provided a signal used in a feedback loop. When the Vaisala RH sensor read lower than the RH setpoint, solenoid valves opened to inject humid air from a gas humidity bottle (model LF-HBA with Nafion® tubing (Fuel Cell Technologies, Inc., Albuquerque, NM, USA). The gas humidity bottle, heated to 140 °F, passed compressed air through Nafion® tubes surrounded by de-ionized water, creating a warm air stream saturated with water vapor. Temperature was controlled by circulation of water through radiators. Figure 2-1 shows the schematic of the ClO_2 fumigation system that was used for these efficacy tests. A similar system was used for the VPHP fumigations by replacing ClO_2 systems with VPHP analogs.

Modified Standard Method (mSM) 4500-ClO_2-B samples were taken every 60 minutes to confirm the concentration of ClO_2 in the test chamber. VPHP concentrations were also verified using wet chemistry methods. Multiple fans were used inside the chamber to provide internal mixing. Pressure relief valves and check valves prevented over-pressurization of the chamber. A room monitor alarmed if there was an accidental release of fumigant. All fumigation gas was directed through a sorbent trap before release into a fume hood.

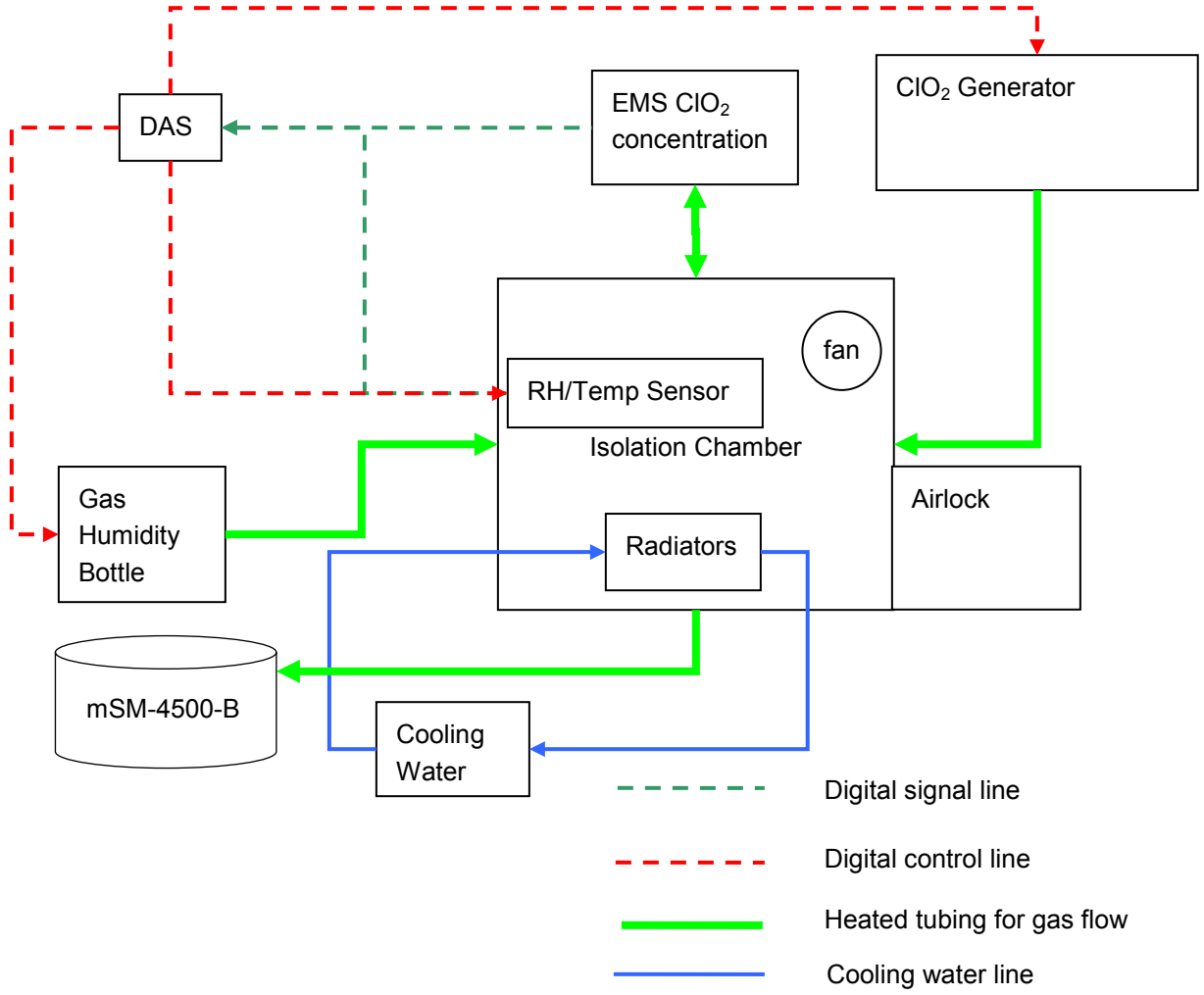

Figure 2-1. Block Diagram of the ClO_2 Fumigation System

The kinetics facility consisted of two chambers connected in series with all the ancillary equipment required for successful estimation of the material demand. The first chamber was used as a constant-concentration feed reservoir to the second kinetic chamber in which all the experiments were performed. A schematic of the kinetic facility is shown in Figure 2-2. The main components added to the existing laboratory setup are described in the following sections.

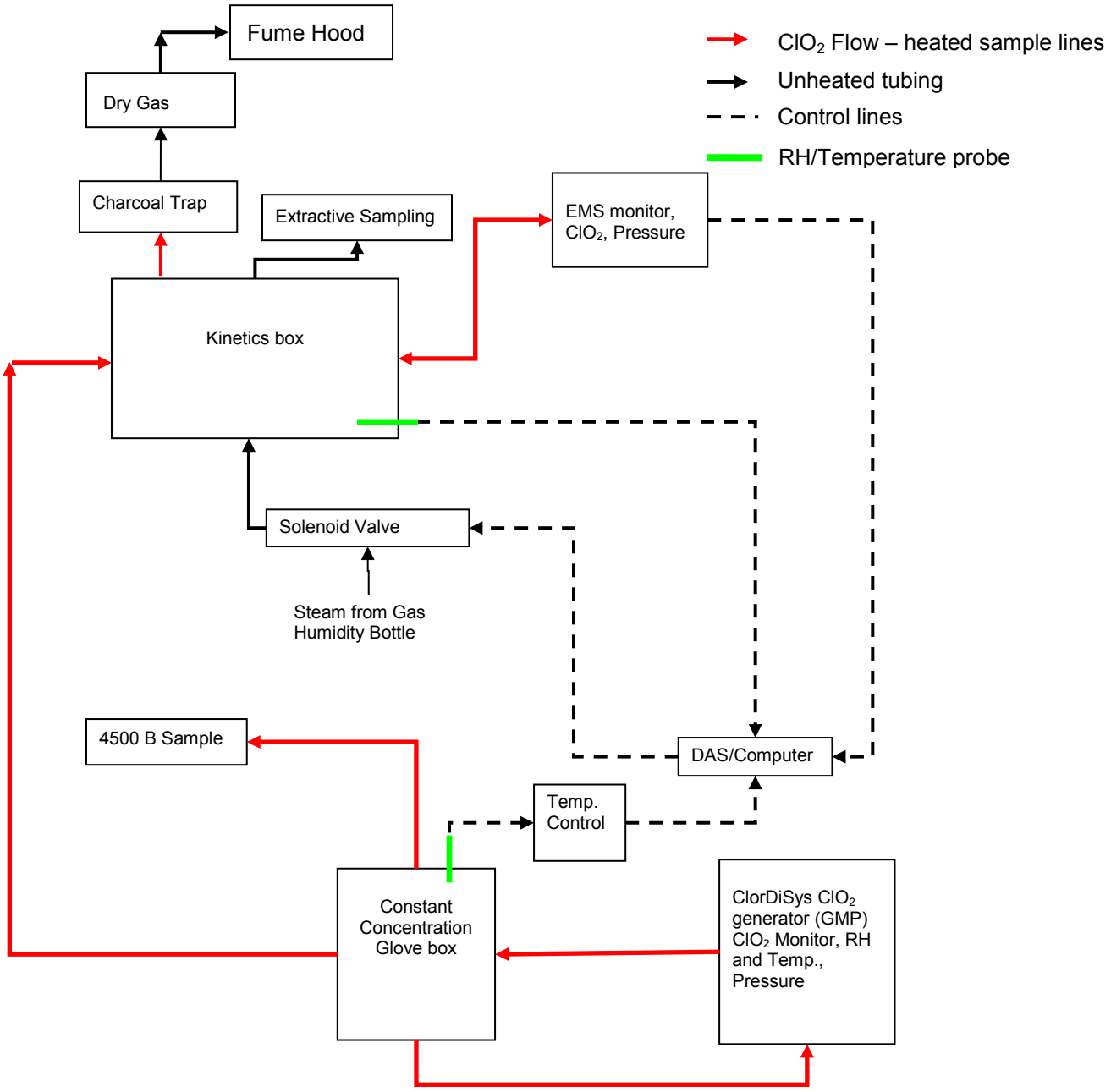

Figure 2-2. Schematic of the Kinetic Facility

2.2 Kinetics Chamber

The kinetics chamber (Figure 2-3) is a stainless steel enclosure with an internal volume of 119 liters, designed for RH and temperature control, and including various ports as shown in Figure 2-2. A fan was installed to aid with internal mixing.

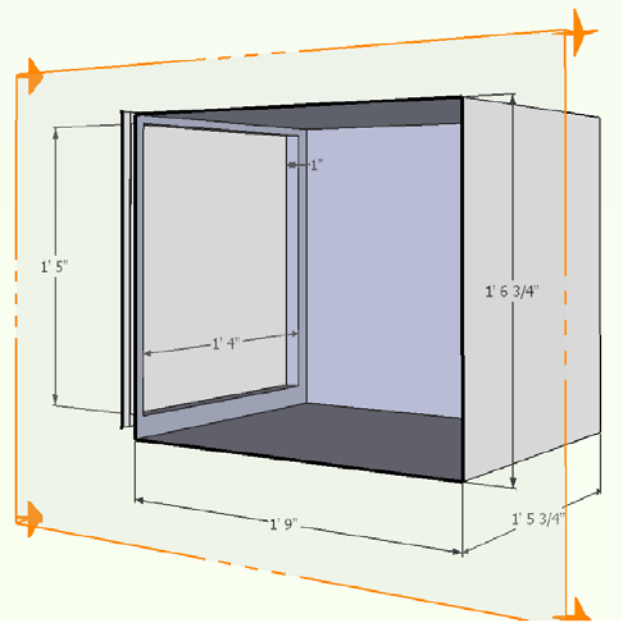

Figure 2-3. Cutaway View of Kinetics Chamber

2.2.1 Air Exchange System

The air exchange was attained by removing air at a set rate from the kinetics chamber. The makeup air came from either the constant concentration glove box (in the case of the exposure and steady-state phases) or from ambient laboratory air (for all other phases). The air exchange rate was constant throughout the entire experiment, set at one exchange per hour to mimic regular air exchanges in an occupied facilities.

2.3 Coupon Preparation

Chunks of subway concrete from the floor of the New York Metropolitan Transit Authority (MTA) Old South Ferry Station were provided to EPA by Department of Homeland Security (DHS) Science and Technology Division via Massachusetts Institute of Technology Lincoln Laboratories (MIT-LL). These chunks were covered in grit, probably from the deconstruction process. The chunks were divided, using dry mechanical methods, into as many coupons as possible that contained a minimum 1.5” x 1.5” square. No information was available on the type of concrete and its age other than that this subway station was established in the early 1900’s.

2.3.1 Coupon Cleaning

A subset of the subway concrete coupons was cleaned using a method adapted from the New York MTA. This was done to assess whether fumigation of a cleaned concrete surface would result in differences in log reduction of spores when compared to fumigation results obtained with subway concrete “as received”. The cleaning solution consisted of Tide® Institutional Formula Floor & All-Purpose Cleaner (4.2 g) mixed in 1.5 liters of hot water to create a 0.28 % solution by weight.

The cleaning procedure consisted of the following seven steps:

1. Place concrete pieces flat in a sink with top surface facing up.
2. Spray with 0.28 % Tide® solution using foaming spray applicator (Trigger Sprayer, Grainger Item # 3U603 on 32 Ounce Spray Bottle, Grainger Item # 3U593).
3. Scrub lightly with 1.5" soft pure-bristle paintbrush.
4. Rinse each piece well under flowing tap water.
5. Stand pieces on edge on a paper towel.
6. Blow dry with dry nitrogen to remove surface water.
7. Let air dry for 24 hours.

A 1.5" square was outlined using a permanent marker (e.g., black or silver Sharpie®) on each coupon before inoculation. This square was used to align the aerosol deposition apparatus (ADA) (see Section 2.4) and served to frame the sampling area.

Additionally, after a coupon was dosed via the procedure detailed in Section 2.4.1.2, the coupon was labeled with the unique identifier. The identification (ID) was written on the side of the coupon using a permanent marker. The stainless steel coupons were pre-labeled on the underside (non-contaminated) side using a black Sharpie®.

Concrete coupons were sterilized with EtO before use by placing coupons in an Andersen EOGas 333 Cabinet (Andersen Products Inc., Haw River, NC, USA) which was set at 50 °C. Typically, an 11 g cartridge of EtO was released into a 22" x 36" diffusion bag containing the items for sterilization. The contents underwent an 18-hour exposure and degassing cycle.

2.4 Method Development I – Material Inoculation

Coupons were inoculated (loaded) with spores of *B. atrophaeus (*formerly *B. globigii)* (American Type Culture Collection (ATCC) 9372) from a metered dose inhaler (MDI).[1] Method development was required to reach the targeted deposition of 1 x 10^6 CFU on a 1.5-inch diameter portion of the concrete surface. Stainless steel coupons were used to quantify CFU for this effort. Coupons were sterilized with ethylene oxide (EtO) before use.

2.4.1 Aerosol Deposition Method Modification

The aerosol deposition method described by Lee et al[1]. and Calfee et al. [2] was modified for targeting a deposition of 1 x 10^6 CFU on a 1.5-inch diameter portion of the concrete surface. Stainless steel coupons were used to quantify CFU for this effort. Stainless steel coupons were autoclaved before use and five coupons were used for each effort. A successful method was required to deliver an average of at least 1 x 10^6 CFU and have a precision of $\pm$0.5 log to have the ability to demonstrate a 6-log reduction in viable spores. These coupons were sampled with sponge wipes according to internal operating procedures.

2.4.1.1 Bacillus Spore Preparation

The test organism for this work was a powdered spore preparation of *Bacillus atrophaeus (*formerly *B. globigii)* (American Type Culture Collection (ATCC) 9372) and silicon dioxide particles. A preparation resulting in a powdered matrix containing approximately 1 x 10^{11} viable spores per gram was prepared by dry blending and jet milling the dried spores with fumed silica particles (Deguss, Frankfurt am Main, Germany). The powdered preparation was loaded into MDIs (Cirrus, Morrisville, NC, USA) and sealed after addition of propellant. Control checks for each MDI as described in Section 2.4.1.2 were included in the batches of coupons contaminated with a single MDI.

2.4.1.2 Coupon Inoculation

Coupons were inoculated (loaded) with spores of *B. atrophaeus (*formerly *B. globigii)* from an MDI. In brief, the inoculation procedure involved placing a round ADA on the top surface of the coupon facing upwards for inoculation (Figures 2-4 and 2-5). The ADA was clamped to the coupon and the MDI was attached to the top of the ADA. A slide was opened, and the MDI was activated. Following inoculation, the slide was closed and the MDI was removed. The spores were allowed to settle for at least 18 hours. This procedure was repeated for each coupon. Inoculation was done on a laboratory bench with coupons placed in a bed of sand to keep the irregular shaped coupons upright.

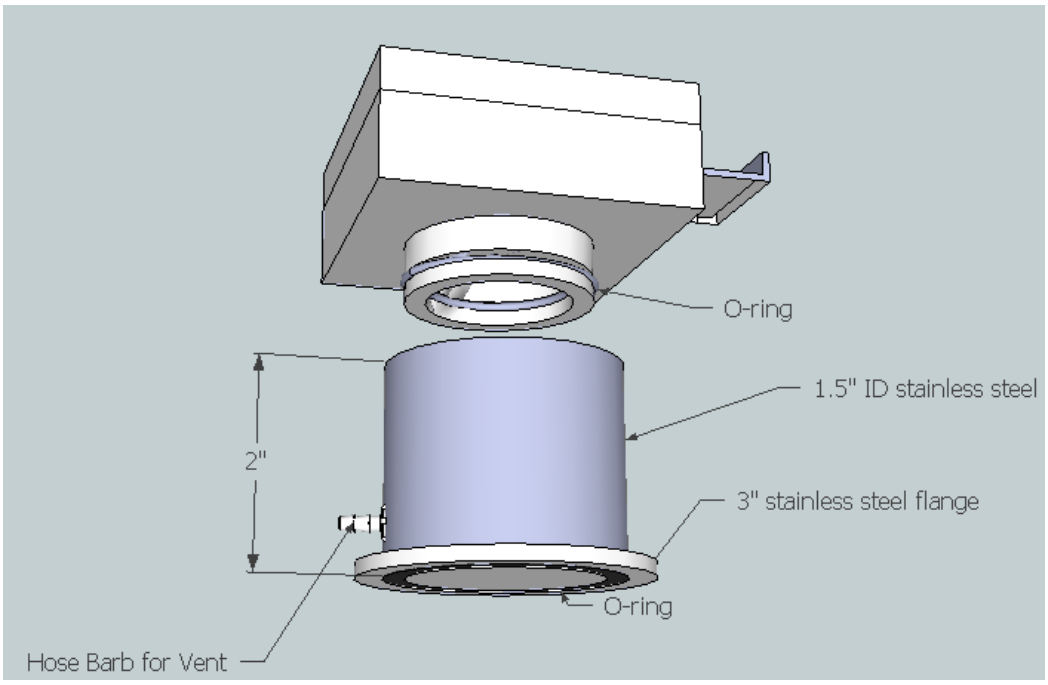

Figure 2-4. Round ADA schematic

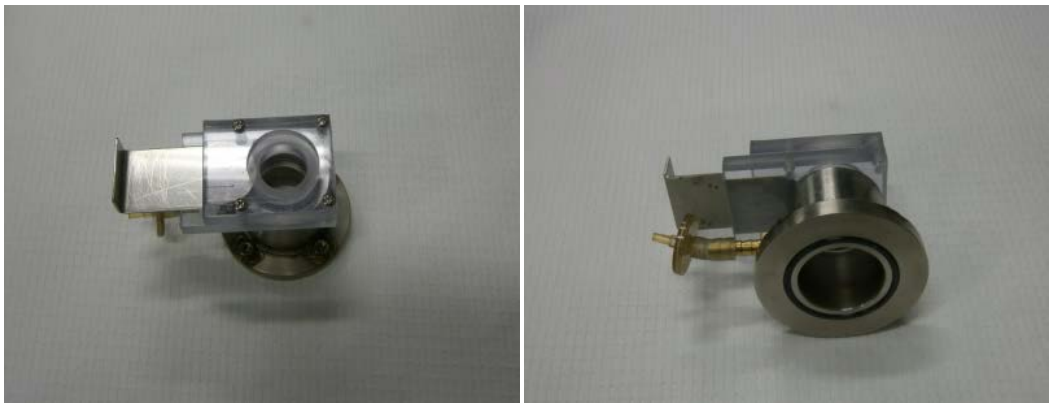

Figure 2-5. Round ADA with O-ring gasket

Each MDI provides 150 discharges before degradation of concentration. The number of discharges per MDI was tracked to ensure that use did not exceed this value. Additionally, in accordance with internal operating procedures, the weight of each MDI was determined after completion of the contamination of each coupon. If an MDI weighed less than 10.5 g at the start of the contamination procedure, the MDI was retired and a new MDI was used. For quality control of the MDIs, pairs of positive control coupons and stainless MDI reference coupons were inoculated as the first, middle, and last coupons within a single group of coupons inoculated by any one MDI within a single test.

A log was maintained for each set of coupons that were dosed via this method. Each record in this log recorded the unique coupon identifier, the MDI unique identifier, the date, the operator, the weight of the MDI before dissemination into the coupon dosing device, the weight of the MDI after dissemination, and the difference between these two weights. The coupon codes were pre-printed on the log sheet prior to the start of coupon inoculation (dosing).

After inoculation, the coupons remained undisturbed for 18 hours to allow the spores to settle, and then the coupons were aseptically transferred to sterilized coupon holders or bins for storage before use.

2.5 Method Demonstration II – Surface Sampling

The modified aerosol deposition method described in Section 2.4 was used to inoculate concrete coupons. Initial sampling methods included sponge wipe,[3,4] 37-mm vacuum filter,[5] and wetted wipe sampling.[6] Nine replicate coupons were inoculated. A successful sampling method would deliver at least 1 x 10^6 CFU and have a precision of ±0.5 log. Inoculation at this level allowed to observe a 6-log reduction in viable spores. Sampling methods were all conducted as per internal operating procedures. Ease of use and cost were factors in determining the method used for sampling. The NHSRC Research Triangle Park (RTP) Biocontaminant Laboratory (hereafter referred to as the NHSRC Biocontaminant Laboratory) quantified the number of viable spores per sample. Following extraction, the resulting samples were plated in triplicate and CFU were enumerated. One method, sponge wipe, was selected for further use as part of the actual decontamination testing.

2.6 Experimental Approach

2.6.1 Fumigation Tests

The modified deposition and sampling methods described in Sections 2.4 and 2.5, respectively, were used to inoculate and sample concrete coupons. Four fumigation scenarios using two fumigation techniques were used to decontaminate triplicate coupons of two types, cleaned and not cleaned. Log reduction was calculated by comparing recovery from fumigated coupons to recovery from coupons that were inoculated but not fumigated (positive controls). The goal was to provide a 6-log reduction in CFU - a benchmark for determining efficacy of a decontamination procedure - under fumigation conditions obtainable in the field. The test matrix is shown in Table 2-1.

Table 2-1. Fumigation Test Matrix

Test ID	Fumigant	Concentration	Exposure Times (hours)	Other Conditions
1	ClO_2	1500 ppmv*	0, 2, 4, and 6	75 % RH, 24 °C
2	ClO_2	500 ppmv	0, 6, 12, and 18	75 % RH, 24 °C
3	VPHP	250 ppmv	0, 1, 2, and 4	< 80 % RH
4	VPHP	150 ppmv	0, 4, 7, and 10	< 80 % RH

* parts per million by volume

Testing was conducted in a glove box and proceeded as follows:

1. Sterilization of all coupons for the test. Coupons were sterilized using EtO. The sterility of the coupons was verified through the use and sampling of laboratory blank control samples as part of each test condition (not fumigated).
2. Inoculation of test and positive control coupons with the procedure developed in Section 2.4.
3. Three (3) test coupons per time point and coupon type and one blank coupon per coupon type (negative controls) were loaded into the glove box.
4. The fumigation with ClO_2 was performed using the ClorDiSys GMP and according to the parameters shown in Table 2-1. The fumigation with H_2O_2 vapor (VPHP) was performed using the STERIS VHP® 1000ED and according to the parameters shown in Table 2-1.
5. After the exposure time was reached, the coupons were transferred to the airlock, where they were aerated for 10 min before removal.
6. Coupons were sampled immediately after removal from the airlock. Samples were transferred to the on-site NHSRC Biocontaminant Laboratory in sterile primary independent packaging within sterile secondary containment containing logical groups of samples. All samples were accompanied by a completed chain of custody (COC) form.

In addition to the steps outlined above, all test activities were fully documented during the activity via narratives in laboratory notebooks, the use of digital photography, and video. The documentation could also include items such as a record of time required for each decontamination step or procedure, any deviations from the test plans, and physical impacts on the materials, among others.

2.6.2 Material Demand Tests

Material demand studies were expected to be conducted for both fumigants. The limited availability of subway concrete coupons did not allow for material demand tests with H_2O_2 fumigant. The decision to only study the ClO_2 material demand was based on previous studies [7,8] that identified the significant material demand of VHP® in the presence of unpainted cinder concrete material. The additional dirt and grime on the concrete surface was not expected to change this high material demand.

The impact of fumigant target concentration on homogeneous and heterogeneous decomposition rates of ClO_2 was assessed during the exposure and aeration phases of a decontamination event. The ClO_2 uptake by the subway concrete was quantified and compared to the homogeneous decomposition of ClO_2 under the same operating conditions. The kinetics chamber was carefully leak checked to avoid

misinterpreting ClO_2 leaks as degradation. If the kinetics chamber did not maintain a vacuum pressure of 2" of water for one minute, corrective actions were taken.

The expected demand of ClO_2 is represented schematically in Figure 2-6 for homogeneous and heterogeneous exposures.

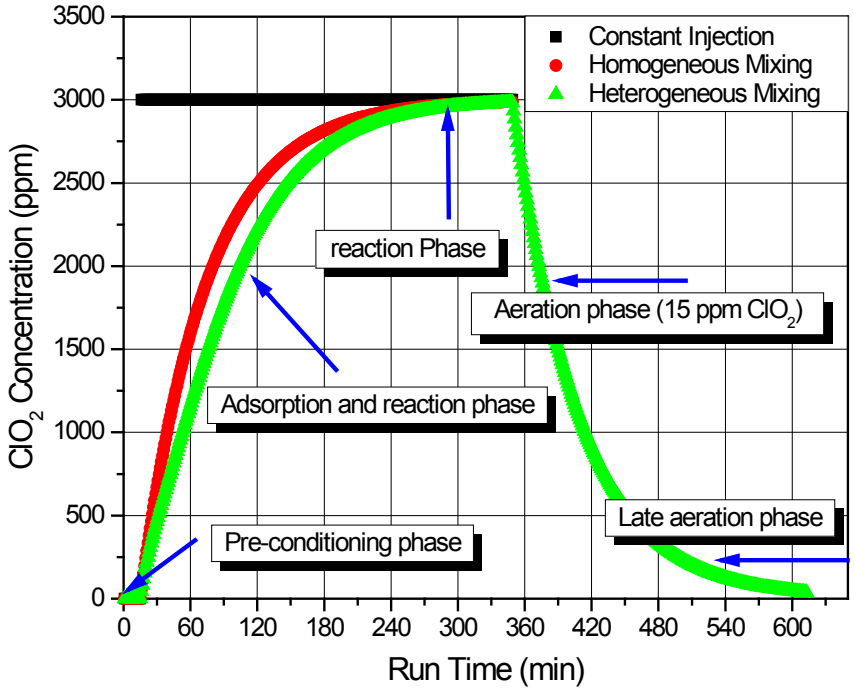

Figure 2-6. Illustration of ClO_2 Breakdown during a Decontamination Event

The overall experimental approach consists of different phases defined as follows:

- Pre-conditioning phase: During this phase, the kinetics chamber was ramped from ambient temperature and RH conditions to the pre-determined temperature and RH conditions for the test.

- Conditioning phase: The kinetics chamber and its contents were maintained at constant temperature and RH before exposure to ClO_2.The conditioning phase did not last more than one minute for this effort.

- Adsorption and reaction phase (Exposure phase): The introduction of ClO_2 into the kinetic chamber began. The concentration of ClO_2 climbed from zero to a steady-state value.

- Reaction Only Phase (Steady-state): The concentration of ClO_2 reached a steady-state maximum. This phase was defined by a change of less than 5 % in the ClO_2 concentration over a period of one hour.

- Aeration Phase: This phase began when the Reaction Only Phase was completed. Ambient air began entering the glove box, reducing the ClO_2 concentration from its state-state value. The aeration phase ended when the concentration in the glove box first fell to below 15 ppm.

- Late Aeration Phase: This phase begins at the end of the Aeration phase and lasts for 12 hours.

The run times and demand rates for each phase shown in Figure 2-6 are presented just for illustration purposes.

During all phases, the air exchange rate was maintained at a constant value. The only change was in the source of the makeup air, either ambient air or the constant concentration of ClO_2 at constant temperature and RH from the glove box. No microbiological samples were collected during this task.

2.6.2.1 ClO_2 Demand in an Homogeneous Environment

The homogeneous test matrix (Tests 1 – 3 in Table 2-2) was designed to determine the extent of the kinetics chamber on the decomposition of ClO_2.

Table 2-2. Kinetics Test Matrix

Test Number	Inlet Concentration (ppm)	RH (%)	Temperature (°F)	Minimum Exposure Time (hours)	Test Material	Surface area of coupons (in^2)
1	250	75	75	36	None	0
2	500	75	75	18		
3	1500	75	75	6		
4	250	75	75	36	Concrete 'As Is'	170
5	500	75	75	18		
6	1500	75	75	6		

Each of the above phases, from pre-conditioning to late aeration, was followed in order. Continuous emission monitoring of ClO_2 concentration, RH, and temperature was performed during all phases of the simulated decontamination event. Extractive samples were also taken during conditioning of the chamber, charging of the chamber, and aeration of the chamber. These samples were analyzed using mSM 4500-ClO_2-B.

2.6.2.2 ClO_2 Demand in the Heterogeneous Environment

The heterogeneous test matrix (Tests 4-6 in Table 2-2) was designed to determine reaction rates of ClO_2 in the presence of grimed subway concrete. Operation of the glove box proceeded as in homogeneous tests; however, the kinetics chamber contained subway concrete of known surface area. Surface area of the coupons was estimated by wrapping the coupons in a single layer of foil and gravimetrically determining the area of the foil used.

Modified SM 4500-ClO_2-B samples were taken every 60 minutes to confirm the concentration of ClO_2 in the test chamber. Pressure relief valves and check valves prevented over-pressurization of the chamber.

The approach to determination of reaction and absorbance values for heterogeneous kinetic tests is described below.

1. For each experiment, the total demand per unit time was calculated by multiplying the difference between the blank concentration and the actual concentration by the flow rate.

$$\text{Total demand (mg/min)} = (C_{(experimental)} - C_{(blank)}) \times F \qquad (2\text{-}1)$$

where: C is concentration (mg/liter) and

F is flow (L/min).

2. Determine the total demand at steady-state concentration, i.e., the highest concentration reached. If the absorbance stage has been completed, the total demand at this stage should equal the heterogeneous reaction rate.

3. Plot the total demand per unit surface area at steady state for all available steady-state concentrations. Use a least-squares model to determine a relationship between concentration and reaction demand:

$$\text{Reaction Demand (mg/min/m}^2) = f(C,t) \qquad (2\text{-}2)$$

4. For each experiment, calculate the reaction demand for each time point based on the relationship determined in Step 3.

5. The adsorption demand may be calculated for each experiment by summing the difference between the actual total demand and the reaction demand for each time point. Because of mixing delays, some individual time points may have negative adsorption demand values. However, the adsorption demand values should average out to a non-negative value.

$$\text{Adsorption demand (mg/run)} = \Sigma(\text{Total demand}_i - \text{Reaction demand}_i) \qquad (2\text{-}3)$$

for each time interval i.

6. Plot the adsorption demand per unit surface area as a function of steady-state concentrations. Use a least-squares model to determine a relationship between adsorption and concentration:

$$\text{Adsorption (mg/m}^2) = f(C) \qquad (2\text{-}4)$$

7. The total demand may be calculated from the following equations:

$$\text{Total Demand (mg)} = \text{Reaction Demand (mg)} + \text{Adsorbance Demand (mg)} \qquad (2\text{-}5)$$

$$\text{Reaction Demand (mg)} = A \times f(C,t) \qquad (2\text{-}6)$$

where: A = total exposed surface area (m^2)

The Reaction Demand component includes a relationship between concentration and time which will be dependent on generation technique. For instance, if the target fumigation was 9000 ppm*hours, the total reaction demand would be a function of the duration of the fumigation.

Adsorbance Demand (mg) = A x f(C) (2-7)

where: C = target concentration (mg/L).

2.7 Sampling and Analytical Procedures

Within a single test, extraction or surface sampling was completed for all blank coupons before sampling of any inoculated coupon was performed. Surface sampling was done either by wipe sampling or vacuum sampling in accordance with the protocols documented below.

Prior to the sampling event, all materials needed for sampling were prepared using aseptic techniques. The materials specific to each protocol are included in the relevant sections below. In addition, general sampling supplies were also needed. A sampling material bin was stocked for each sampling event. The bin contained enough wipe sampling and vacuum sampling kits to accommodate all required samples for the specific test. Additional kits of each type were also included for backup. Sufficiently prepared packages of gloves and bleach wipes were included in the bin. Extra gloves and wipes were also included. A sample collection bin was used to transport samples back to the on-site NHSRC Biocontaminant Laboratory. The exterior of the transport container was decontaminated by wiping all surfaces with a bleach wipe or towelette moistened with a solution of pH-adjusted bleach (pAB) prior to transport from the sampling location to the NHSRC Biocontaminant Laboratory.

Fumigation conditions were reached before coupons were exposed to the fumigant. Fumigation generation equipment was enclosed inside a spray booth, which necessitated long fumigant injection tubes. However, the tube length did not seem to affect fumigation conditions. Room alarms were present for both fumigants.

The glove box used for fumigation was insulated from light and heat. Following fumigation, coupons were aerated for at least ten minutes before sampling. ADAs were sterilized using EtO before each use.

2.7.1 Sampling Strategy

The primary objective was to study the impact of grime on fumigation effectiveness as to decontaminate concrete subway surfaces. The effectiveness is measured by the determination of the LR calculated per Section 1.2.1. Sampling of positive controls was compared to post-decontamination sampling of test sections for this study. Since current surface sampling techniques are intrusive, they will also remove viable spores from the surface of the coupon. Positive control coupons were inoculated on the same day and analyzed on the same day as test coupons but were not decontaminated.

For the material demand task, mass balances of ClO_2 fumigant were performed in real time on the kinetic chamber by constantly monitoring the inlet and outlet concentration of ClO_2 as well as the flow rate. To

confirm the readings of the photometers (which also respond to Cl_2 gas), wet chemistry samples based on mSM 4500-ClO_2-B were collected every hour.

2.7.2 Sampling Points

Each sampling method was used on the surface of coupons inoculated with approximately 1 x 10^6 spores. For each inoculation event, additional samples were collected from stainless steel surfaces as MDI control samples. Wipe samples or vacuum samples were collected by sampling within a 1.5" x 1.5" sampling template pre-printed on the coupons.

ClO_2 and VPHP concentration measurements were collected from fumigation and kinetics chambers (ClO_2 only for kinetics study). Fans in the chambers provided mixing, ensuring that the measurements were representative.

2.7.3 Sampling Frequency

Table 2-3 lists the frequency of all samples for the fumigation tests.

Table 2-3. Sample Frequency

Sample Type	Quantity	Frequency	Process Type or Location	Purpose
Test Coupon	3 per coupon type and fumigation condition	1 set per fumigation	Fumigated	To determine the number of viable spores after fumigation
Negative Control Coupon	1 per coupon type	1 per fumigation	Not fumigated	To determine extent of cross-contamination and/or the sterility of coupons
Procedural Blank Coupon	1 per coupon type	1 per fumigation (earliest time point)	Fumigated	To determine extent of cross-contamination during fumigation
Positive Control Coupon	3 per coupon type, inoculated as the first, middle, and last coupons	1 per inoculation	Not fumigated	To determine the number of viable spores recoverable from the coupons
MDI Control Coupons (stainless steel)	3 per inoculation event, inoculated immediately before each positive control coupon	1 per inoculation	Not fumigated	To determine the number of viable spores deposited onto the coupons and to assess the stability of the MDI
NHSRC Biocontaminant Laboratory Material Blanks	3 per material	Once per use of material	NA	To demonstrate sterility of extraction and plating materials
H_2O_2 Monitor	1	Real time during H_2O_2 fumigations	Glove box	To determine exposure experienced by the coupons
H_2O_2 Wet Chemistry Samples	Duration dependent	1 every 2 hours during fumigation	Glove box	To validate operation of H_2O_2 monitor
H_2O_2 Wet Chemistry Sample Blank (laboratory air)	1	1 per H_2O_2 fumigation	NA	Procedure for sample collection and titration
ClO_2 Monitor	1	Real-time during ClO_2 fumigations	Glove box	To determine exposure experienced by the coupons
mSM 4500-ClO2-B Wet Chemistry Samples	Duration-dependent	Once every 60 minutes	Glove box	To validate operation of ClO_2 real-time monitors

Sample Type	Quantity	Frequency	Process Type or Location	Purpose
ClO_2 Wet Chemistry Sample Blank (laboratory air)	1	1 per ClO_2 fumigation	NA	To demonstrate blank value of mSM 4500-ClO_2-B
RH/Temperature	1	Logged every 10 seconds	Glove box	To determine environmental conditions during fumigations

NA = not applicable.

Table 2-4 lists critical and non-critical measurements for each sample.

Table 2-4. Critical and Non-Critical Measurements

Sample Type	Critical Measurements	Non-critical Measurement
Test Coupon	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Negative Control Coupon	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Positive Control Coupon	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Field Blank Coupons	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
Lab Blank Coupons	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
NHSRC Biocontaminant Laboratory Material Blanks	Plated volume, incubation temperature, extracted volume, CFU	Storage time, storage temperature
H_2O_2 Monitors	H_2O_2 concentration	NA
H_2O_2 Wet Chemistry Samples	Volume collected, volume of titrant used	Temperature of meter box, time for collection
H_2O_2 Wet Chemistry Sample Blank	Volume collected, volume of titrant used	Temperature of meter box, time for collection
ClO_2 Monitors	ClO_2 concentration	NA
mSM 4500-ClO_2-B Wet Chemistry Samples	Volume collected, volume of titrant used	Temperature of meter box, time for collection
ClO_2 Wet Chemistry Sample Blank	Volume collected, volume of titrant used	Temperature of meter box, time for collection
RH/Temperature	RH and temperature during fumigation	NA

NA = not applicable.

Table 2-5 lists the frequency of all samples for the material demand tests.

Table 2-5. Sample Frequency for Material Demand Tests

Sample Type	Quantity	Frequency	Location	Purpose
Coupon Surface Area	1	Each coupon	Laboratory	To determine the total surface area exposed to allow calculations of demand per surface area
ClO_2 Monitor	1 per chamber	Real-time during ClO_2 fumigations	Constant source chamber and kinetics chamber	To determine exposure experienced by the coupons
mSM 4500-ClO_2-B Wet Chemistry Samples	Duration dependent	Once every 60 minutes	Constant Source chamber and kinetics chamber	To validate operation of ClO_2 real-time monitors
ClO_2 Wet Chemistry Sample Blank (laboratory air)	1	1 per ClO_2 fumigation	Laboratory air	To demonstrate blank value of mSM 4500-ClO_2-B
RH/Temp	1	Logged every 10 seconds	Kinetics chamber	To determine environmental conditions during fumigations
Background Demand Test	1 per constant concentration	1	Laboratory	To determine material demand of empty kinetics chamber
Material Demand Tests	1 per constant concentration	1 per coupon type	Laboratory	To determine material demand of grimed concrete

Table 2-6 lists critical and non-critical measurements for each sample collected during the material demand task.

Table 2-6. Critical and Non-Critical Measurements for Material Demand Task

Sample Type	Critical Measurements	Non-critical Measurement
ClO_2 Monitors	ClO_2 concentration	
mSM 4500-ClO_2-B Wet Chemistry Samples	Volume collected, volume of titrant used	Temperature of meter box, time for collection
ClO_2 Wet Chemistry Sample Blank	Volume collected, volume of titrant used	Temperature of meter box, time for collection
RH/Temp	RH and temperature during fumigation	
Background Demand Test	Flow rate, real time concentration, exposure time	
Material Demand Tests	Flow rate, real time concentration, surface area present, mass of concrete present, exposure time	

Digital video was collected during representative events (inoculation, fumigation, and sampling). Photographs of selected material coupons with any visible change due to the sampling procedure were taken after the completion of the sampling.

2.7.4 Statistical Approach

Section 1.2.1 details the methods for determining the efficacy or LR of a fumigation technique for each coupon location. The Student's t-test was used to evaluate whether a variable such as fumigation

duration had an effect on efficacy. The LR was also plotted against variables such as measured fumigant concentration to determine any relationship.

2.7.5 Sampling Procedures

2.7.5.1 Polyester-Rayon Blend (PRB) Wipe Sampling

PRB wipe sampling is typically used for small sample areas and is effective on nonporous, smooth surfaces such as ceramics, vinyl, metals, painted surfaces, and plastics.[3] PRB wipe sampling was used for concrete samples during method devlopment as per internal operating procedures. The general approach is that a moistened sterile nonwoven PRB pad is used to wipe a specified area to recover bacteria, viruses, and biological toxins. The protocol that was used in this project has been adopted from that provided by Busher et al.[3] and Brown et al.[4] Wipe samples were extracted in 20 mL Phosphate Buffered Saline with 0.05 % TWEEN® 20 (PBST) and subjected to serial tenfold dilution and spread-plating as per internal operating procedures.

2.7.5.2 37 mm Vacuum Sampling

For concrete samples during method devlopment, 37-mm vacuum sampling using polytetrafluoroethylene (PTFE) filters (0.3 micron pore size) was used.[5] A 1.5" x 1.5" square was vacuumed on each coupon. The 37-mm samples were extracted as per internal operating procedures and subjected to tenfold serial dilution and spread-plating.

2.7.5.3 Sponge Wipe Sampling

Sponge wipe sampling was used for concrete samples during method development and as part of the fumigation testing. Sponge wipe samples were collected using the following two patterns: (1) using the flat side of the sponge wipe, the surface was sampled using horizontal S-strokes, covering the entire template area; and (2) the sponge wipe was then flipped over to the opposite side to sample the surface in a vertical pattern, covering the entire template area. This is an abbreviation of the sampling method described in detail in the study Rose et al,[6] which was designed to sample a larger area than the one used for this study. Sponge wipe samples were extracted in 90 mL PBST and subjected to tenfold serial dilution and spread-plating.

2.7.5.4 Wet Chemistry Samples

The ClO_2 extractive samples were collected hourly according to internal operating procedures during ClO_2 fumigations. The H_2O_2 extractive samples were collected every two hours during VPHP fumigations.

2.7.5.5 Coupon Spore Enumeration

The NHSRC Biocontaminant Laboratory quantified the number of viable spores per sample (vacuum and wipe samples). PBST was used as the extraction buffer for all sample types. After the appropriate extraction procedure, as described in the sections to follow, the buffer was subjected to a five-stage serial dilution (10^{-1} to 10^{-5}). The resulting samples were plated in triplicate and incubated overnight at 35 °C ± 2°C. Following incubation, CFU were enumerated as per internal operating procedures. The PBST was prepared according to internal operating procedures.

The extraction procedure used to recover spores was varied depending upon the different matrices (PRB wipes, sponge sticks, or vacuum filters) according to internal operating procedures. Other procedures are described in the following subsections. Samples that have fewer than the reportable limit of 30 CFU/plate in the undiluted sample underwent filter plating and re-plating. While there are no EPA-approved methods for spore enumeration from surfaces, the use of positive control samples as the baseline for log reduction calculations includes a built-in verification of the deposition and enumeration methods.

2.7.5.6 Extractive ClO_2 Analysis

ClO_2 concentration in an air sample was determined by titration. Briefly, sodium thiosulfate is used to reduce iodine oxidized by the ClO_2. The titration goes from a yellow solution to a colorless endpoint.

2.7.5.7 Extractive H_2O_2 Analysis

H_2O_2 concentration in an air sample was measured by titration. Briefly, potassium permanganate is used to oxidize H_2O_2 dissolved under acidic conditions. The titration goes from a colorless solution to the first pink tinge.

2.8 Sample Handling and Custody

2.8.1 Preventing Cross-Contamination during Sampling

Sampling poses an additional significant opportunity for cross-contamination of samples. In an effort to minimize the potential for cross-contamination, several management controls were followed.

- In accordance with aseptic technique, a sampling team was utilized, made up of a “sampler,” a “support person,” and a “sample handler.”
- The sample handler was the only person to handle ADAs or material coupons during the sampling event.
- The sampler handled only the sampling media and the support person handled all other supplies. The sampler sampled the surface according to internal operating procedures.
- The collection medium (e.g., PRB wipe or 37-mm cassette) was placed into a sample container that was opened, held and closed by the support person.
- The sampler placed the 37-mm nozzle directly into the small conical tube with sterile gloves. The tube was opened, held and closed by the support person.
- The sealed sample was handled only by the support person.
- All of the following actions were performed only by the support person, using aseptic technique:
    - The sealed bag with the sample was placed into another sterile plastic bag that was then sealed; that bag was then decontaminated using a bleach wipe.
    - The double-bagged sample was then placed into a sample container for transport.
    - The exterior of the transport container was decontaminated by wiping all surfaces with a bleach wipe or towelette moistened with a solution of hypochlorite prior to transport from the sampling location to the NHSRC Biocontaminant Laboratory.
- After the sample was placed into the container for transport, the sample handling team placed the

sampled material in pAB for decontamination and eventual disposal.

- The sampling crew then changed their gloves in preparation for working with the next sample.

Additionally, and equally important, the order of sampling was as follows: (1) all blank coupons; (2) decontaminated coupons; and (3) positive control coupons. This order ensured that coupons were handled in an order from lowest level of contamination to the most.

2.8.2 Preventing Cross-Contamination during Analysis

General aseptic laboratory technique was followed and is embedded in the standard operating procedures (SOPs) and MOPs used by the NHSRC Biocontaminant Laboratory to recover and plate samples. The SOPs and MOPs document the aseptic technique employed to prevent cross-contamination.

Additionally, the order of analysis (consistent with the above) was as follows: (1) all blank coupons; (2) all decontaminated coupons; and (3) all positive control coupons.

2.8.3 Representative Samples

This work was meant to explore the efficacy of fumigants within subway systems. The concrete coupon materials were taken from an actual subway system. The fumigation conditions are considered representative of conditions that could be met in the field.

2.8.4 Sample Quantities

The sample quantities are outlined in Table 2-2. Concrete coupon quantities were limited by the finite amount of New York subway concrete available for testing.

2.8.5 Sample Containers for Collection, Transport, and Storage

For each PRB wipe sample, the primary containment was an individual sterile 50 mL conical tube. Secondary containment was sterile sampling bags. The primary containment of the 37-mm sample was a sterile 10” x 5.5” sample bag. The inlet tube for the 37-mm sample was primarily contained in a separate sterile 15 mL conical tube. The secondary containment of the inlet tube and 37-mm cassette was separate sterile sampling bags. The primary containment of the sponge wipe was a Seward stomacher bag (Seward Limited, Worthing, West Sussex, UK), secondarily contained in an individual sterile sampling bag. All biological samples from a single test were then placed in a sterilized container. After samples were placed in the container for storage and transport to the NHSRC Biocontaminant Laboratory, the container was wiped with a towelette saturated with at least 5000 parts per million (ppm) hypochlorite solution by weight. A single container was used for storage in the decontamination laboratory during sampling and for transport to the NHSRC Biocontaminant Laboratory.

2.8.6 Sample Preservation

Following transfer to the NHSRC Biocontaminant Laboratory, all samples were stored at 4 °C ± 2 °C until they were analyzed. Samples were stored no longer than five days before the primary analysis. A typical holding time, prior to analyses, for most biological samples was two days. All samples were allowed to equilibrate to room temperature for one hour prior to analysis.

2.8.7 Sample Archiving

All samples and diluted samples were archived for at least two weeks following completion of analysis. This time allowed for review of the data to determine if any re-plating of selected samples was required. Samples were archived by maintaining the primary extract at 4 °C ± 2°C in a sealed extraction tube.

3 Results and Discussion

3.1 Method Development I

The 1.5” round ADA deposition method demonstrated the ability to deliver the desired concentration of at least 1×10^6 CFU per coupon with a better than 0.5 log precision. This ensures that a 6-log reduction in viable spores can be demonstrated. Recovery from sponge wipes of the four replicate stainless steel samples is shown in Table 3-1. The table shows repeatable recovery of 1.3×10^7 CFU per sample with a relative standard deviation (RSD) of 30 %.

Table 3-1. Recovery from Stainless Steel Coupons following Prototype Deposition Method

Replicate	CFU/sample	Log CFU	Average CFU/sample	SD	RSD
1	9.56E+06	7.0	1.34E+07	4.06E+06	30 %
2	1.52E+07	7.2			
3	1.05E+07	7.0			
4	1.82E+07	7.3			

SD = Standard deviation.

Based on these results, the method describing the 1.5” ADA deposition method, was adopted for all subsequent inoculations.

3.2 Method Development II

Three sampling methods were evaluated to repeatedly recover CFU from concrete samples inoculated using the 1.5” round ADA deposition method. The results are displayed in Table 3-2 and Figure 3-1.

Table 3-2. Recovery of Various Concrete Sampling Methods

Sample	Average	Maximum	Minimum	RSD
Stainless Controls	1.18E+07	1.55E+07	8.78E+06	29 %
Sponge Wipe	9.27E+06	1.05E+07	6.83E+06	23 %
Wetted Wipe	1.08E+07	1.77E+07	4.31E+06	62 %
37-mm Vacuum	2.35E+06	3.27E+06	1.31E+06	42 %

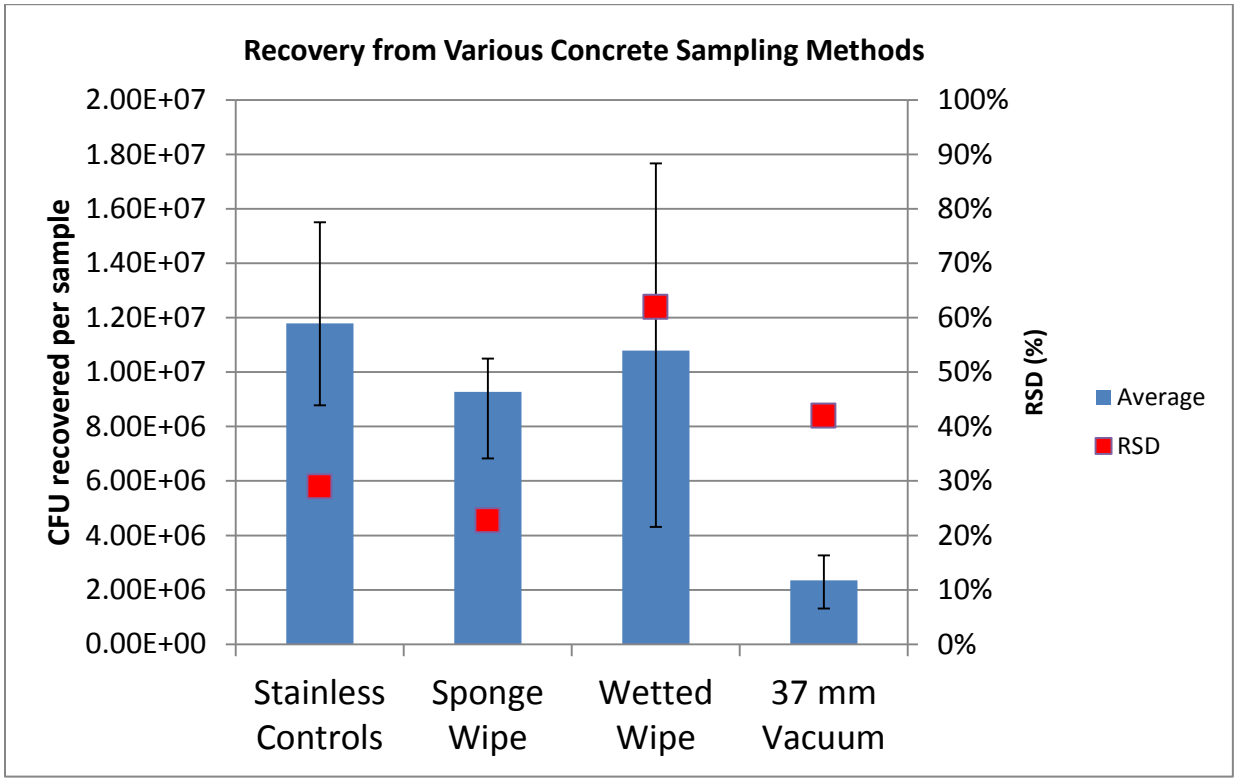

Figure 3-1. Recoveries of Various Concrete Sampling Methods

Results from the sponge wipe of stainless control coupons is shown for reference. Wetted wipe samples had a large variability, with a relative standard deviation (RSD) of 62 %. Wetted wipe samples are also not recommended because the sampler has direct contact with the sample. The 37-mm vacuum samples recovered only 20 % of the spores compared to the stainless steel control samples. For these reasons, the sponge wipe was chosen as the preferred concrete sampling method for subsequent fumigation tests.

3.3 ClO_2 Fumigations

As discussed in Section 2.1, two measurement methods were used for determining ClO_2 concentration: a real–time photometer and the periodic wet chemistry method based on mSM 4500-ClO_2-B. For the duration of the wet chemistry samples, photometer samples were also collected. Wet chemistry values were 9% higher than average photometer reading at the time of sampling. ClO_2 concentrations reported for the remainder of this report, when based on real-time photometer data, are standardized to the wet chemistry value using this correction.

Conditions during the two ClO_2 fumigations are shown in Table 3-3. Average and standard deviation values were calculated for the duration of each exposure.

Table 3-3. Fumigation Test Matrix

1500 ppmv Test				500 ppmv Test			
Target Conditions: 1500 ppmv, 75 % RH, 24 °C				Target Conditions: 500 ppmv, 75 % RH, 24 °C			
Exposure Time (h)	**ClO_2 (ppmv)**	**Temp (°C)**	**RH (%)**	**Exposure Time (h)**	**ClO_2 (ppmv)**	**Temp (°C)**	**RH (%)**
2.5	1570 ± 40	23.3 ± 0.02	75.0 ± 0.1	6	570 ± 40	23.4 ± 0.1	76 ± 1
4	1570 ± 40	23.3 ± 0.02	75.0 ± 0.1	12	550 ± 40	23.4 ± 0.1	75 ± 0.3
6	1570 ± 40	23.3 ± 0.03	75.0 ± 0.1	18	550 ± 40	23.4 ± 0.2	76 ± 1

Microbiological results from the 1500 ppmv ClO_2 test are shown in Figure 3-2.

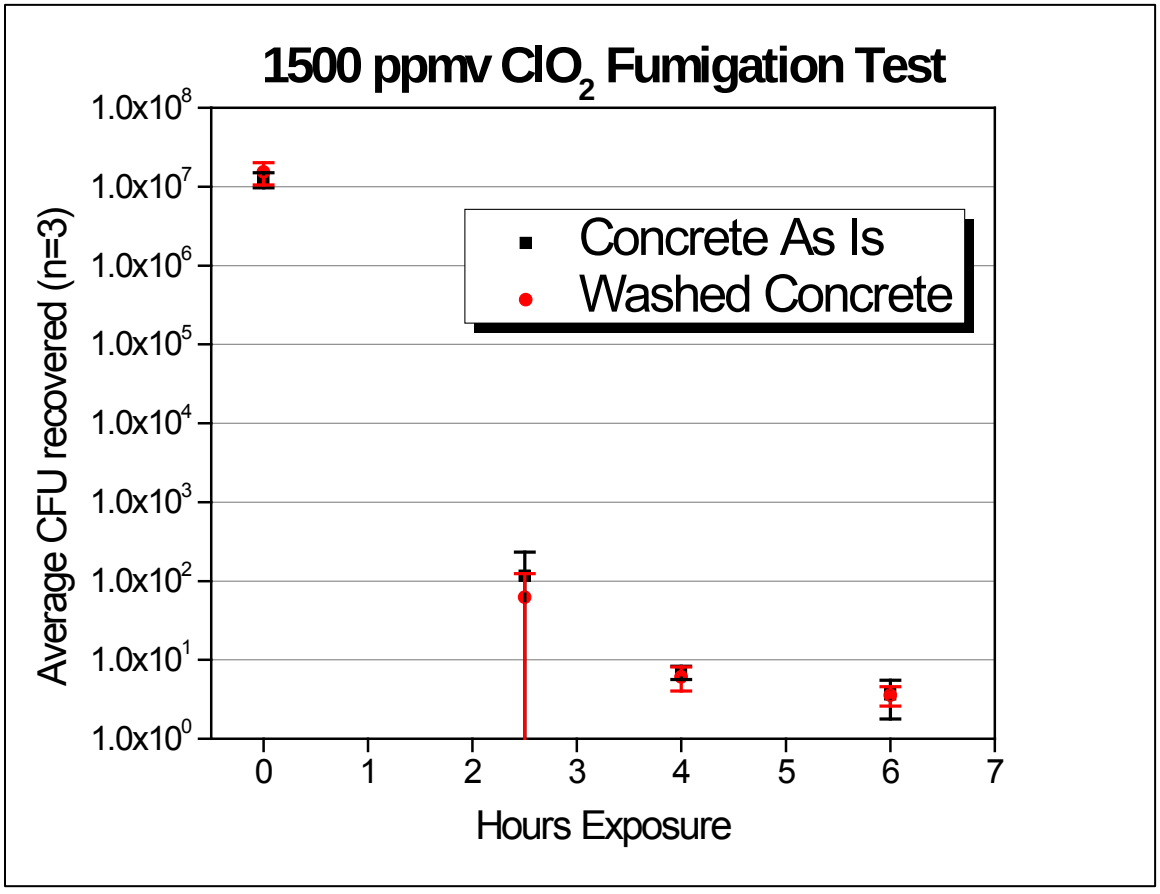

Figure 3-2. Average CFU Recovered from Concrete following Exposure to 1500 ppmv ClO_2.

Some spores (1 CFU each on one of the three unwashed concrete coupons and 1 CFU on one of the three washed concrete coupons) were recovered after 6 hours of exposure. These values are very close

to the detection limit of the method. Washed and unwashed concrete behaved similarly, with no statistical difference between spores recovered from the two types of coupon preparations (t = 0.95). Figure 3-3 shows the same results in terms of log reduction.

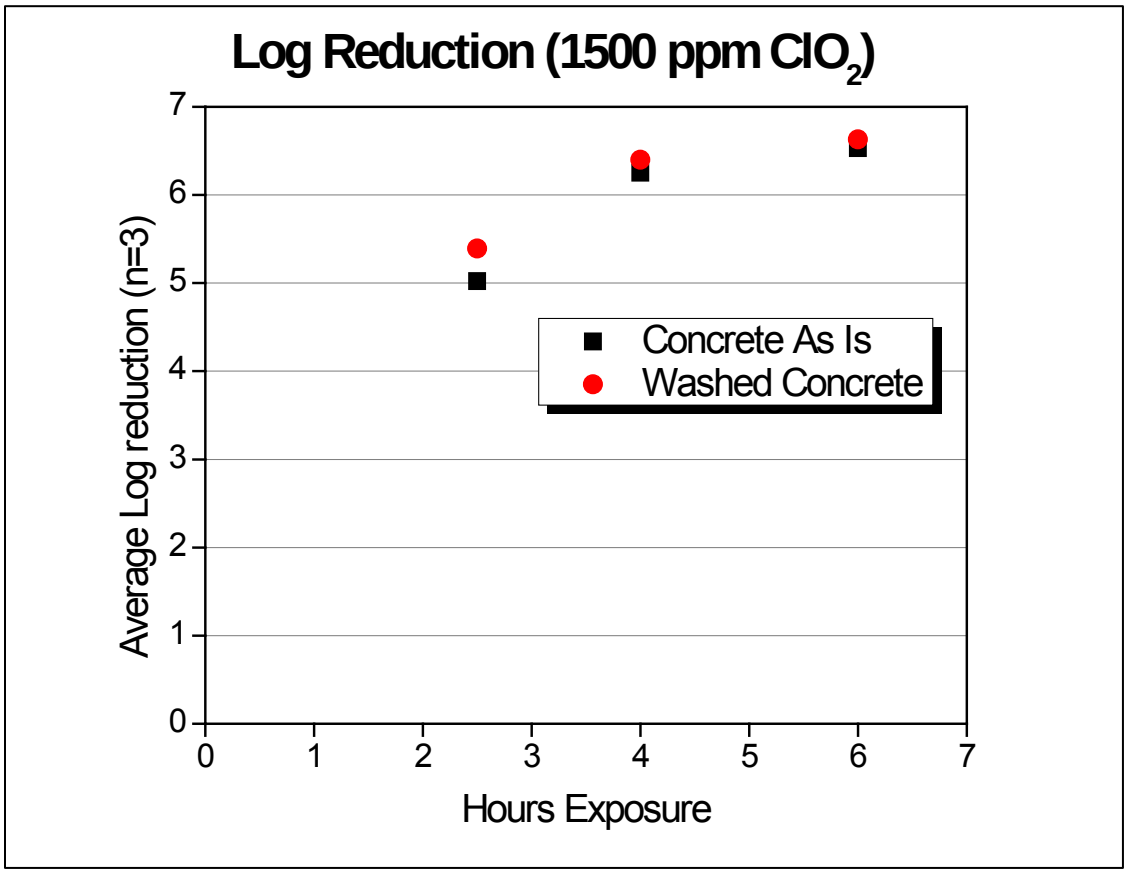

Figure 3-3. Log Reduction of Spores Recovered from Concrete following 1500 ppmv ClO_2

Both the 4- and 6-hour fumigation durations indicate a greater than 6-log reduction.

The second test (Figure 3-4) was conducted to look at the efficacy when using a lower ClO_2 concentration. This test extended to longer exposure times to duplicate concentration × time (CT) exposure levels.

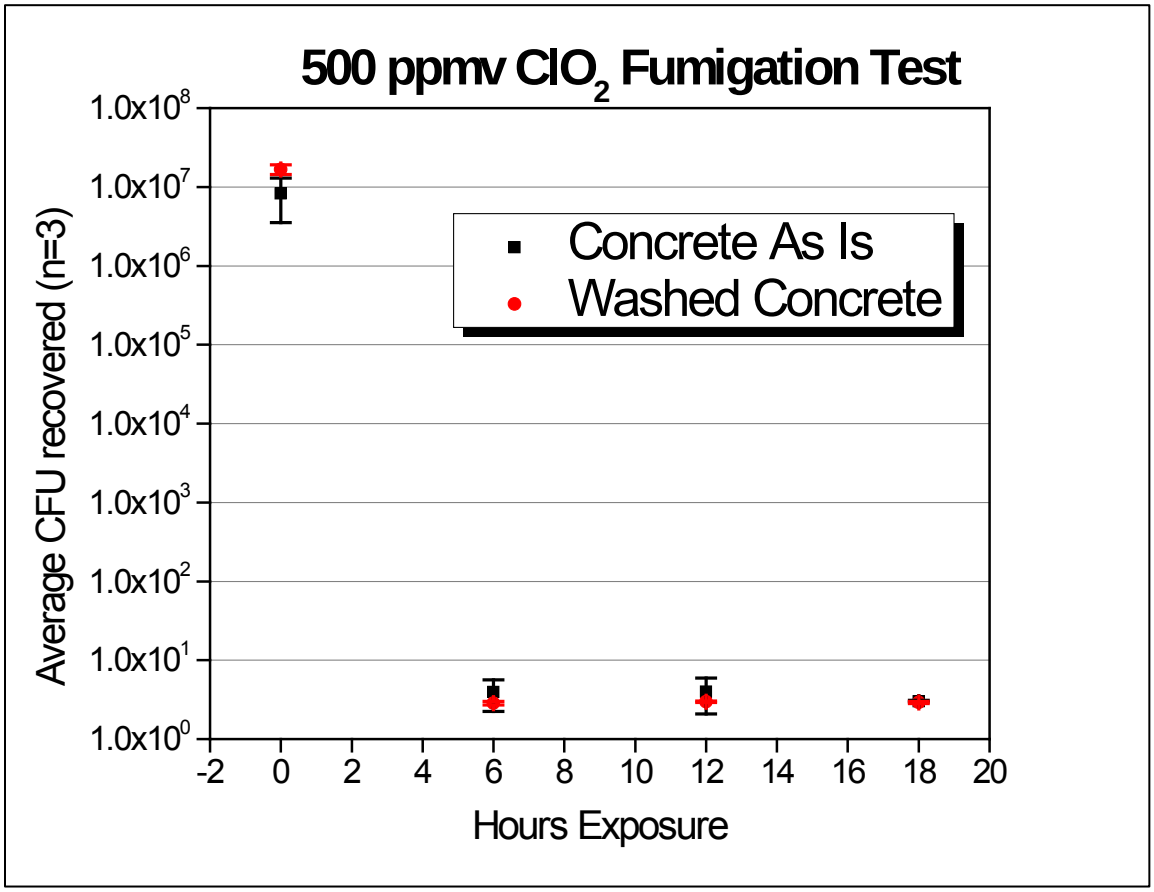

Figure 3-4. Average CFU Recovered from Concrete following Exposure to 500 ppmv ClO_2

Very few spores were recovered from all three of the non-zero time points of the 500 ppmv ClO_2 fumigation. No spores were recovered from any of the washed concrete coupons. Of the unwashed concrete, one spore was recovered from one of the three replicate coupons for the first two time points. A total of two spores were recovered from all 18 fumigated coupons. Based on this limited dataset, the results show no significant difference in efficacy for the cleaned and uncleaned concrete subway surfaces. All three time points provided a 6-log reduction. However, only the longest exposure (18 hours) provided complete decontamination (no detection).

3.4 H_2O_2 Fumigations

Conditions during the H_2O_2 fumigations are shown in Table 3-4. Average and standard deviation values were calculated for the duration of each exposure.

Table 3-4. H_2O_2 Fumigation Test Matrix

250 ppmv test				150 ppmv test			
Target Conditions: 250 ppmv H_2O_2, < 80 % RH				Target Conditions: 150 ppmv, < 80 % RH			
Exposure Time (h)	**H_2O_2 (ppmv)**	**Temp (°C)**	**RH (%)**	**Exposure Time (h)**	**H_2O_2 (ppmv)**	**Temp (°C)**	**RH (%)**
1	250 ± 4	17.0 ± 0.1	21 ± 1	4	151 ± 13	20.0 ± 0.5	11 ± 2
2	250 ± 4	17.1 ± 0.1	21 ± 1	7	151 ± 12	20.0 ± 0.3	11 ± 2
4	250 ± 11	17.2 ± 0.2	21 ± 1	10	151 ± 11	20.0 ± 0.5	11 ± 2

Microbiological recovery for the 250 ppmv H_2O_2 is shown in Figure 3-5.

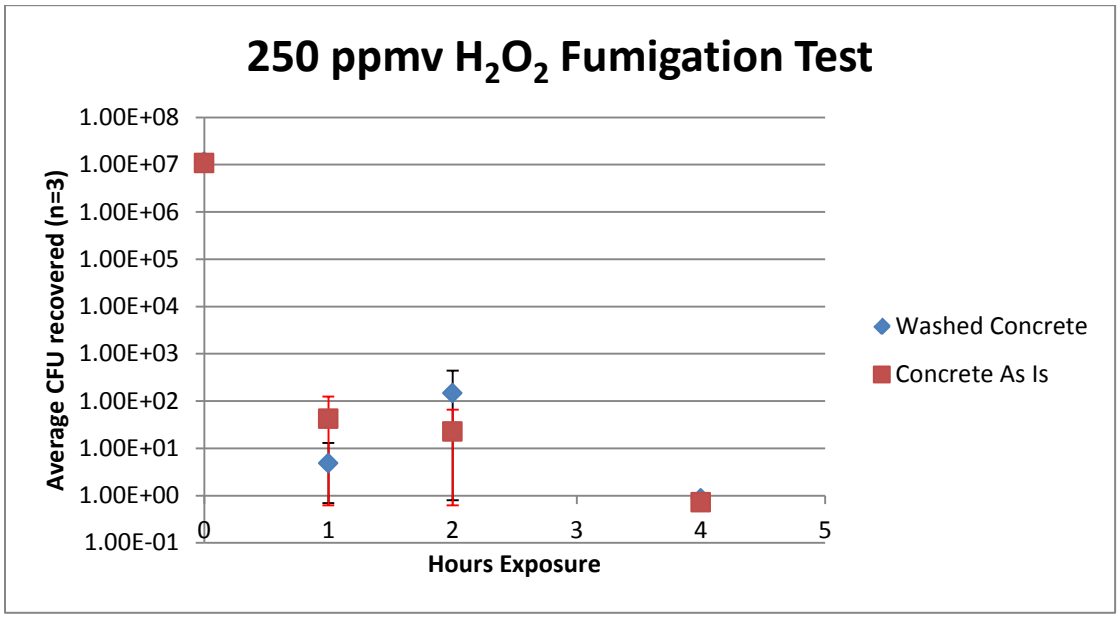

Figure 3-5. Average CFU recovered from Concrete following Exposure to 250 ppmv VPHP

While there are differences in the response of washed and unwashed concrete coupons, these differences are not statistically significant based on the observed variation in the average CFU recovered for each set of coupons. One- and two-hour fumigation of both concrete types produced some non-detect coupons. All coupons from the four-hour exposure at 250 ppm were non-detect. Only the 4-hour fumigation at 250 ppmv VPHP produced an average LR over 6.

These results are in contrast to the fumigation at 150 ppmv VPHP. No tested exposure time at the lower VPHP concentration provided a 6-log reduction. Average recovery is shown in Figure 3-6.

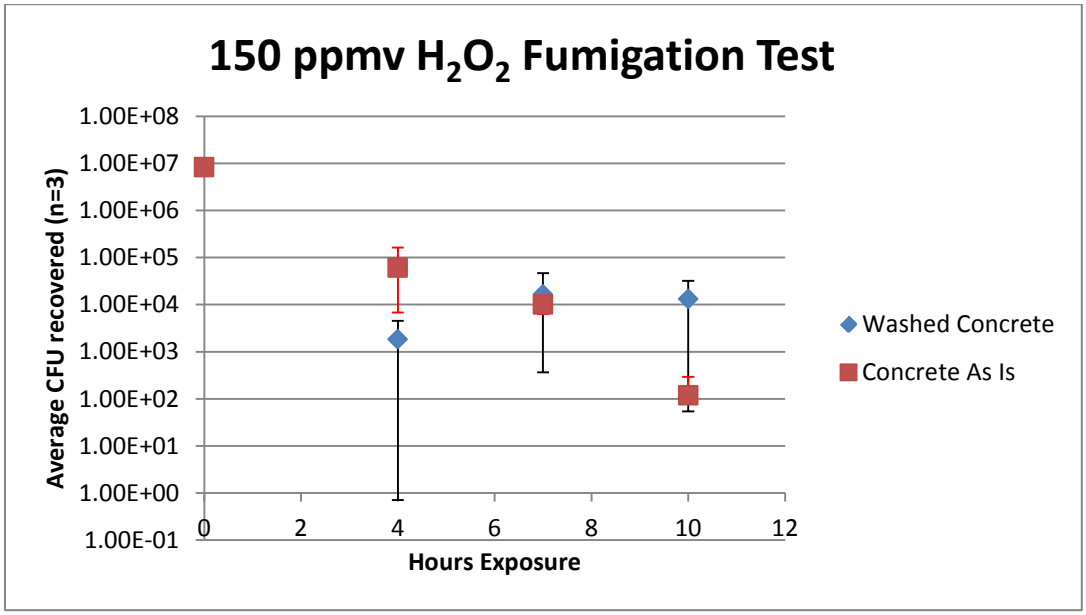

Figure 3-6. Average Recovery from Coupons after Exposure to 150 ppmv VPHP

As in the 250 ppm test, there does not seem to be an effect of grime on concrete before VPHP fumigation. The apparent benefit at the 4-hour exposure is contradicted by the results of the 10-hour exposure.

Table 3-5 shows the average LR for both tests in terms of CT. Even at much higher CT values, LR from the 250 ppmv test was lower. This investigation suggests there is no clear relationship between CT and efficacy for VPHP fumigation of concrete. For concrete, high concentrations of H_2O_2 may be needed, unless very long exposure times are used. An extrapolation of log reduction over exposure time at 150 ppm (see Figure 3-7) suggests that a 6 LR may be reached after 13 hours, compared to 6 hours at 250 ppm.

Table 3-5. Average LR of Washed and Unwashed Concrete

250 ppmv Test			150 ppmv Test		
ppm*hours	Washed Concrete	Concrete 'As Is'	ppm*hours	Washed Concrete	Concrete 'As Is'
246	6.38	5.41	605	3.65	2.13
497	4.89	5.68	1062	2.69	2.91
989	7.12	7.18	1511	2.79	4.85

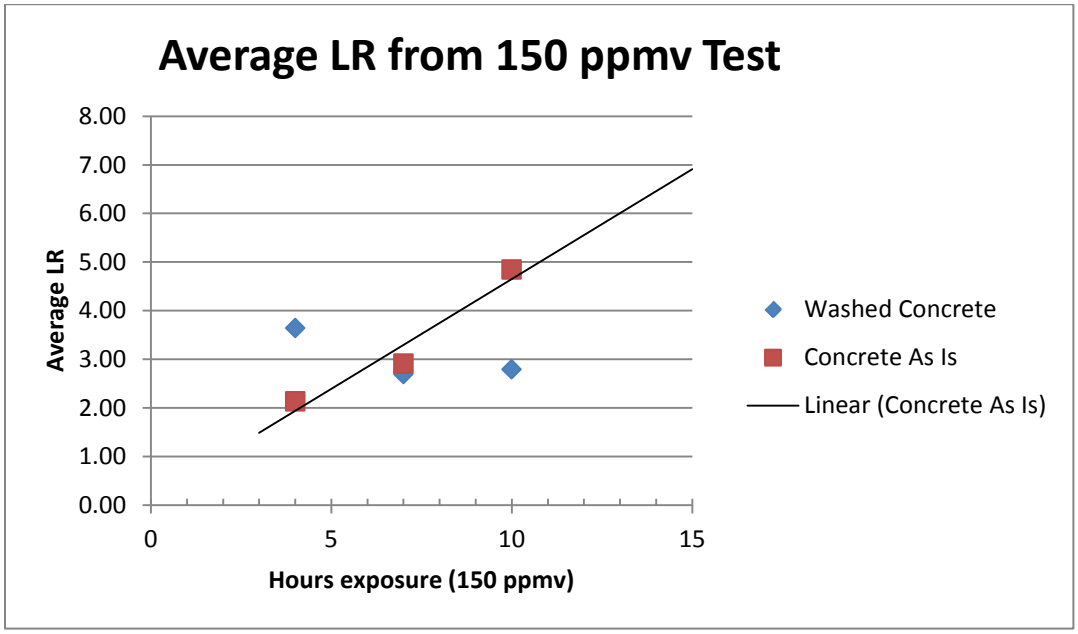

Figure 3-7. Average LR from Coupons after Exposure to 150 ppmv VPHP

3.5 Material Demand of ClO_2

Material demand tests were conducted only on grimed concrete with ClO_2 fumigant due to limited availability of (grimed) subway concrete samples. Material demand tests were not considered for VHP® as the impact of grime was considered to be minimal in comparison to the known high demand in the presence of concrete.[8]

The ClO_2 fumigant tests showed very little material demand by the grimed concrete, on the same order of magnitude as the empty kinetics chamber (blank or detection limit value). This is consistent with the minimal demand for clean concrete.[7] Figure 3-8 shows the ClO_2 concentration in the empty chamber (no demand) and actual test with grimed concrete coupons present during the 250 ppm test. Inlet concentration varied due to poor control at this lower setpoint.

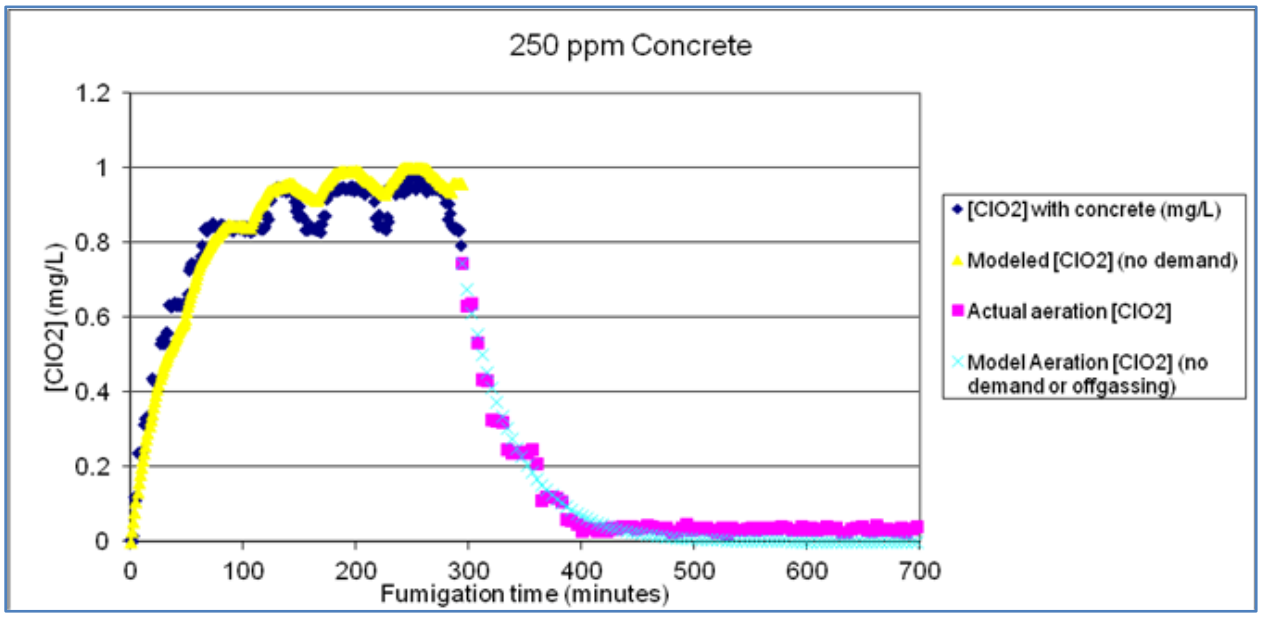

Figure 3-8. Material Demand Test at 250 ppm ClO_2

No absorbance is seen, as demonstrated by no lag time in the rise of the concentration in the presence of the grimed concrete. Reaction rates are also very low, as seen in the relative equivalence in the steady-state concentration. Figure 3-9 shows the reaction rates of the empty (blank) kinetics chamber and of the kinetics chamber including concrete with a surface area of approximately 170 in^2 as a function of ClO_2 concentration.

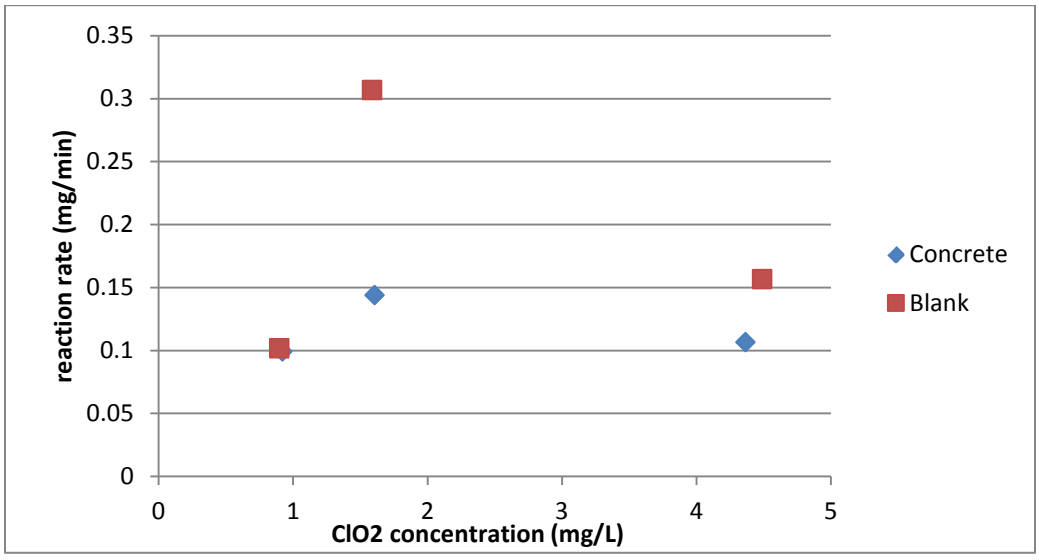

Figure 3-9. Steady-state Reaction Rates of Kinetics Chamber with and without Concrete Samples

There is no apparent difference between the reaction rate with the concrete and without, so the reaction rate was set to zero for the absorbance calculations (see Section 2.6.2.2). These adsorption rates are shown in Figure 3-10 and again suggest no adsorption.

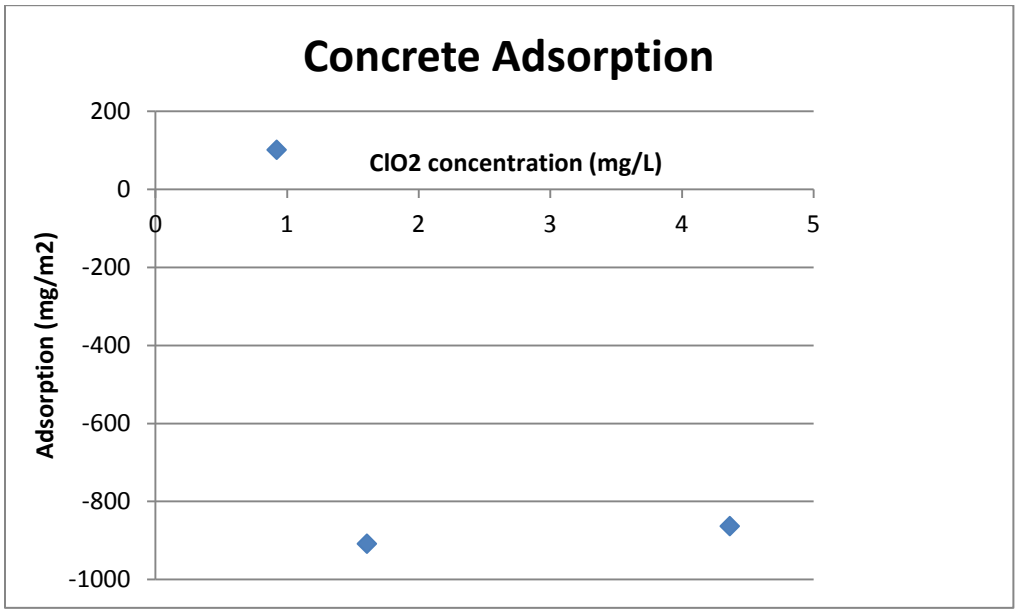

Figure 3-10. Concrete Adsorption as a Function of Concentration.

Negative adsorption rate values are indicative of the limited ability to measure the small difference between adsorption characteristics of the empty chamber and the chamber with concrete coupons. It is possible that some material demand could have been detected with larger amounts of concrete in the chamber, but tests were done with 10 pieces with a surface area of 170 in^2 due to the limited availability of the subway concrete. Neither reaction nor absorbance components of ClO_2 demand were detected for grimed concrete.

4 Quality Assurance

This project was performed under an approved Category III Quality Assurance Project Plan (QAPP) titled *Interaction of Fumigation with Realistic Surfaces from Subway System* (June 2013) and an addendum for Task 4 – Material Demand (February, 2014), both available upon request.

4.1 Sampling, Monitoring, and Analysis Equipment Calibration

There were standard operating procedures for the maintenance and calibration of all laboratory and NHSRC Biocontaminant Laboratory equipment. All equipment was verified as being certified calibrated or having the calibration validated by EPA's Air Pollution Prevention and Control Division (APPCD) on-site (Research Triangle Park, NC) Metrology Laboratory at the time of use. Standard laboratory equipment such as balances, pH meters, biological safety cabinets and incubators were routinely monitored for proper performance. Calibration of instruments was done at the frequency shown in Tables 4-1 and 4-2. Any deficiencies were noted. The instrument was adjusted to meet calibration tolerances and recalibrated within 24 hours. If tolerances were not met after recalibration, additional corrective action was taken, possibly including recalibration or/and replacement of the equipment.

Table 4-1. Sampling and Monitoring Equipment Calibration Requirements

Equipment	Calibration/Certification	Expected Tolerance
Meter Box	Volume of gas is compared to NIST-traceable dry gas meter annually	± 2 %
RH Sensor	Compare to 3 calibration salts once a week	± 5 %
Stopwatch	Compare against NIST* Official U.S. time at http://nist.time.gov/timezone.cgi?Eastern/d/-5/java once every 30 days.	± 1 min/30 days
Clock	Compare to office U.S. Time @ time.gov every 30 days	± 1 min/30 days
Temperature Sensor	Compare to independent NIST thermocouple annually	± 2 % full scale
Buret	Gravimetric verification of volume performed annually	± 1 %

* National Institute of Standards and Technology

All titrants have a certification of analysis with NIST-traceable concentration values.

Table 4-2. Analysis Equipment Calibration Frequency

Equipment	Calibration Frequency	Calibration Method	Acceptance Criteria
Pipettes	Annually	Gravimetric	± 1 % target value
Burets	Annually	Gravimetric	± 1 % target value
Pressure Manometer	Annually	Compared to NIST-traceable Heiss gauge	± 3 % reading
Incubator thermometers	Annually	Compared to NIST-traceable thermometer	± 0.2 °C
Scale	Before each use	Compared to Class S weights	± 0.01 % target

4.2 Data Quality

The primary objective of this research was to evaluate the impact that dirt and grime, as present on unpainted subway concrete, have on fumigation efficacy. Secondary objectives included determining which sampling procedure provides better recovery from grimed and cleaned concrete, and characterizing subway material before and after cleaning using a prescribed method from the New York City MTA. This section discusses the Quality Assurance/Quality Control (QA/QC) checks (Section 4.3) and Acceptance Criteria for Critical Measurements (Section 4.4) considered critical to accomplishing the project objectives.

The QAPP in place for this project was followed with deviations noted as follows:

- Subway concrete coupons were sterilized with EtO rather than being autoclaved as specified in the QAPP to remove any effect of the autoclave heat on any grime present on the concrete.

- Fumigation conditions listed in the test matrix (Table 2-1) were determined after the QAPP was approved.

- Concrete availability prevented testing of washed concrete as part of the material demand testing.

4.3 QA/QC Checks

Samples were maintained to ensure their integrity. Samples were stored away from standards or other samples that could cross-contaminate them. While the size and shape of the concrete coupons varied, the size of the inoculation and sampling area did not.

Supplies and consumables were acquired from reputable sources and were NIST-traceable when possible. Supplies and consumables were examined for evidence of tampering or damage upon receipt and prior to use, as appropriate. Supplies and consumables showing evidence of tampering or damage were not used. All examinations were documented and supplies were appropriately labeled. Project

personnel checked supplies and consumables prior to use to verify that they meet specified task quality objectives and did not exceed expiration dates.

Quantitative standards do not exist for biological agents. Quantitative determinations of organisms in this investigation did not involve the use of analytical measurement devices. Rather, CFU were enumerated manually and recorded. Critical QC checks are shown in Table 4-3. The acceptance criteria were set at the most stringent level that could be achieved routinely and are consistent with the data quality objectives described in Section 4.4. Positive controls and procedural blanks were included along with the test samples in the experiments so that well-controlled quantitative values were obtained. Background checks were also included as part of the standard protocol. Replicate coupons were included for each set of test conditions. Qualified, trained, and experienced personnel ensured data collection consistency. When necessary, training sessions were conducted by knowledgeable parties, and in-house practice runs were used to gain expertise and proficiency prior to initiating the research.

Table 4-3. QA/QC Sample Acceptance Criteria

Sample Type	Purpose	Acceptance Criteria	Corrective Actions	Frequency
Negative Control Coupons	Determine extent of cross-contamination	No detectable spores	Values on test coupons of the same order of magnitude will be considered to have resulted from cross-contamination	1 per sample type
Field Blank Coupons	Verify the process of moving coupons does not introduce contamination	No detectable spores	Determine source of contamination and remove	1 per test
Laboratory Blank Coupons	Verify the sterility of coupons following autoclaving	No detectable spores	Determine source of contamination and remove	3 per test
Laboratory Material Coupons	Verify the sterility of materials used to analyze viable spore count	No detectable spores	Determine source of contamination and remove	3 per material per test
Blank Tryptic Soy Agar Sterility Control (plate incubated, but not inoculated)	Controls for sterility of plates	No observed growth following incubation	All plates are incubated prior to use, so any contaminated plates will be discarded	Each plate
Positive Control Coupons	Used to determine the extent of inoculation on the coupons	1×10^6 CFU ± 0.5 log	Outside target range: discuss potential impact on results; correct loading procedure for next test and repeat depending on decided impact	3 per coupon type per test
mSM 4500-ClO_2-B Wet Chemistry	Validate ClO_2 concentration measurements	15 % of photometric reading	Repeat	1 per hour
H_2O_2 Wet Chemistry	Validate H_2O_2 concentration measurements	65 % of electrochemical reading	Check calibration of electrochemical sensor	2 per hour

Sample Type	Purpose	Acceptance Criteria	Corrective Actions	Frequency
Fumigation Extraction Blank Samples	Validated baseline of extractive techniques	Non-detect	Obtain new reagents	1 per test
Puffing Control Coupons	Used to determine drift in the MDI	The CFU recovered from the first set of positive controls must be within 0.5 log of the second set of positive controls	Reject results and repeat test	3 per inoculation
Replicate Plating of Diluted Microbiological Samples	Used to determine variability in CFU counts	The reportable CFU of triplicate plates must be within 100 %. Reportable CFU are between 30 and 300 CFU per plate	Re-plate sample	Each sample
Post-test Calibration of RH Sensors (Vaisala, Helsinki, Finland)	Used to validate sensor operation	The post-test calibration check readings must be within 5 % of target reading	Reject results. Repeat test as deemed appropriate	1 per test

4.4 Acceptance Criteria for Critical Measurements

The Data Quality Objectives (DQOs) are used to determine the critical measurements needed to address the stated objectives and specify tolerable levels of potential errors associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all of the project objectives:

- enumeration of spores on the surface of the concrete coupons;
- concentration measurements to characterize the fumigation conditions.

Table 4-4 lists the quantitative acceptance criteria for critical measurements. Failure to provide a measurement method or device that met these goals would result in a rejection of results derived from the critical measurement. For instance, if the plated volume of a sample is not known (i.e., is not 100 % complete), then that sample is invalid. If a mSM 4500-B sample for ClO_2 is lost or does not meet the criteria for other reasons, then another should be collected to take its place. In contrast, for the real-time H_2O_2 measurements, some missing data would not invalidate a test.

Table 4-4. Critical Measurement Acceptance Criteria

Critical Measurement	Measurement Device	Accuracy	Precision	Detection Limit	Completeness
Plated volume	Pipette	± 2 %	± 1 %	NA	100 %
CFU/plate	Hand counting	± 10 % (between 2 counters)	± 10 %	1 CFU	100 %
ClO_2 concentration	mSM 4500-B	± 15 % of photometric value	± 5 %	10 ppm	90 %
H_2O_2 concentration	ATI sensor	±10 % range	± 5 %	10 ppm	90 %
Fumigation Time	Timer	± 1 second	± 1 second	1 second	100 %
RH/Temp of Fumigation	Vaisala HMD40Y	± 5 %	± 3 %	NA	90 %
Surface Area	Milligram Balance	$\pm$ 0.001 g	$\pm$ 0.001 g	$\pm$ 0.001 g	100 %
Flow Rate	Dry Gas Meter	±1 %	±1 %	1 % full scale	90 %
Surface Area of Concrete	Balance	$\pm$ 0.01 g	$\pm$ 0.01 g	0.01 g	100 %

Plated volume critical measurement goals were met. All pipettes are calibrated yearly by an outside contractor (Calibrate, Inc.).

Plates were quantitatively analyzed (CFU/plate) using a manual counting method. For each set of results (per test), a second count was performed on 25 percent of the plates with significant data (data found to be between 30-300 CFU). All second counts were found to be within 10 percent of the original count.

There are many additional QA/QC checks used to validate microbiological measurements. These checks include samples that demonstrate the ability of the NHSRC Biocontaminant Laboratory to culture the test organism, as well as to demonstrate that materials used in this effort do not themselves contain spores. The checks include:

- Negative control coupons: sterile coupons placed in glove box and fumigated;
- Field blank coupons: sterile coupons carried to fumigation location but not fumigated;
- Laboratory blank coupons: sterile coupons not removed from NHSRC Biocontaminant Laboratory;
- Laboratory material coupons: includes all materials, individually, used by the NHSRC Biocontaminant Laboratory in sample analysis;
- Positive control coupons: coupons inoculated but not fumigated; and
- MDI inoculation control coupons: stainless steel coupons puffed at beginning, middle, and end of each inoculation campaign, not fumigated, to assess the stability of the MDI during the inoculation operation.

The ClO_2 photometer calibrations were checked prior to each test and were within the factory specifications during each fumigation. The primary ClO_2 measurements were the mSM 4500-B extractive samples. The accuracy and precision of the titration equipment were checked using a single-point NIST-traceable standard solution. The Analytical Technologies, Inc. (ATI) H_2O_2 sensor was the primary measurement device for H_2O_2 fumigations. The accuracy and precision of this instrument was assessed by placing the unit in the headspace of a known concentration of H_2O_2. All DQI goals were met for all measurements in Table 4-4.

4.5 Data Quality Audits

This project was assigned QA Category III and did not require technical systems or performance evaluation audits.

4.6 QA/QC Reporting

QA/QC procedures were performed in accordance with the QAPP for this investigation.

5 Summary and Recommendations

The primary objective of this investigation was to determine the effect of real-world grime on the efficacy of ClO_2 and VPHP fumigations of subway concrete. A method was developed using MDIs to inoculate small 1.5" coupons of concrete. Three surface sampling methods were tested (sponge wipe, wetted wipe, and 37- mm vacuum filter); all three methods were comparable for recovery from stainless steel coupons, but the sponge wipe method had higher and more repeatable recovery.

Fumigation of subway concrete using ClO_2 resulted in a greater than 6-log reductions in *Bacillus* spores for 1500 ppmv ClO_2, 75% RH and ≥ 4 h contact time or 500 ppmv ClO_2, 75% RH and ≥ 6 h contact time (shortest contact time tested). Results suggest that fumigation of washed subway concrete does not result in different efficacy values than fumigation of the unwashed subway concrete. The observed differences are minimal and statistically not significant.

No differences were observed in feed concentration and the time required to reach the target ClO_2 fumigation conditions with and without grimed subway concrete present. Hence, no ClO_2 demand was observed for this grimed subway concrete (approximately 170 in^2 surface area of concrete material).

Fumigation with H_2O_2 as generated using Steris VHP® technology resulted in a greater than 6-log reduction in *Bacillus* spores on subway concrete for 250 ppm H_2O_2, 20% RH and a ≥ 4 h contact time. The 6-log reduction in spores could not be reached, even at the longest tested contact time (10 h) at 150 ppmv H_2O_2 concentration. Observed differences in log reduction between washed and unwashed subway concrete following VHP® fumigation were not statistically significant.

No material demand studies were conducted for the VHP® fumigant. Previous research efforts indicated a high demand due to decomposition of hydrogen peroxide at the concrete surface. A recent full scale VHP® fumigation demonstration, as conducted as part of the Bio-response Operational Testing and Evaluation (BOTE) project,[9] also indicated that material demand is significant. In that study, VHP® fumigation did not achieve and maintain a 250 ppmv H_2O_2 concentration for 1.5 h in a 8000 ft^2 facility.

This study showed that three of the four fumigation conditions using two different fumigants provided conditions which demonstrated greater than 6-log reduction of *Bacillus* spores. No impact was observed in the log reduction of spores in the presence of dirt and grime on these subway concrete surfaces. Nevertheless, the high demand observed for VPHP in the presence of substantial amounts of concrete may make achieving the necessary VPHP fumigation conditions challenging. Other fumigants may need to be considered as alternatives to chlorine dioxide fumigation of concrete. Fumigants such as methyl bromide and formaldehyde have, however, so far not been evaluated for fumigation of grimed concrete.

6 References

1. Lee, S. D.; Ryan, S. P.; Snyder, E. G., Development of an aerosol surface inoculation method for *Bacillus* spores. *Applied and Environmental Microbiology* **2011,** *77* (5), 1638-1645.
2. Calfee, M. W.; Lee, S. D.; Ryan, S. P., A rapid and repeatablemethod to deposit bioaerosols on material surfaces. *Journal of Microbiological Methods* **2013,** *92*, 375-380.
3. Busher, A. Noble-Wang, J.;Rose L., Surface sampling, in *Sampling for Biological Agents in the Environment*. Emanuel, P.; Roos, J.W.; Niyogi, K., Eds. ASM Press: Washington, DC, 2008; p 95-131.
4. Brown, G. S.; Betty, R. G.; Brockmann, J. E.; Lucero, D. A.; Souza, C. A.; Walsh, K. S.; Boucher, R. M.; Tezak, M.; Wilson, M. C.; Rudolph, T., Evaluation of a wipe surface sample method for collection of *Bacillus* spores from nonporous surfaces. *Applied and Environmental Microbiology* **2007,** *73* (3), 706-710.
5. Calfee, M. W.; Rose, L. J.; Morse, S.; Mattorano, D.; Clayton, M.; Touati, A.; Griffin-Gatchalian, N.; Slone, C.; McSweeney, N., Comparative evaluation of vacuum-based surface sampling methods for collection of Bacillus spores. *Journal of Microbiological Methods* **2013,** *95* (3), 389-396.
6. Rose, L. J.; Hodges, L.; O'Connell, H.; Noble-Wang, J., National validation study of a cellulose sponge wipe-processing method for use after sampling Bacillus anthracis spores from surfaces. *Applied and Environmental Microbiology* **2011,** *77* (23), 8355-8359.
7. U.S. EPA. Material Demand Studies: Interaction of Chlorine Dioxide Gas with Building Materials. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-08/091, 2008
8. U.S. EPA. Material Demand Studies: Materials Sorption of Vaporized Hydrogen Peroxide. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-10/002, 2010
9. U.S. EPA. Bio-response Operational Testing and Evaluation (BOTE) Project. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-13/168, 2013.

EPA
United States
Environmental Protection
Agency

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT NO. G-35

Office of Research and Development (8101R)
Washington, DC 20460