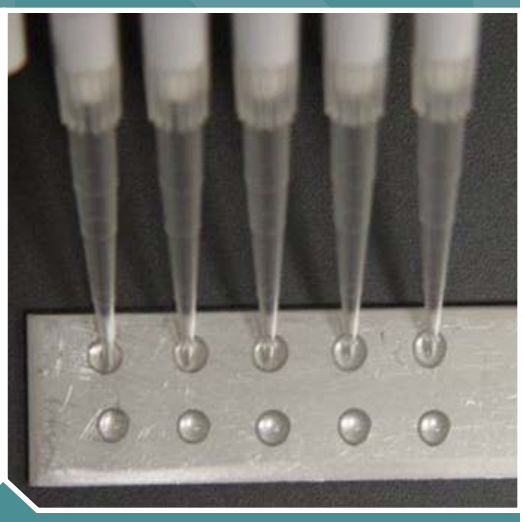
EPA 600/R-11/124 | October 2011 | www.epa.gov/ord

United States
Environmental Protection
Agency

Evaluating a Decontamination Technology Based on the Electrochemical Generation of Anolyte Solution against B. anthracis Spores

TECHNOLOGY EVALUATION REPORT



Office of Research and Development
National Homeland Security Research Center

EPA/600/R-11/124
October 2011

Technology Evaluation Report

Evaluating a Decontamination Technology Based on the Electrochemical Generation of Anolyte Solution against *B. anthracis* Spores

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

Disclaimer

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's National Homeland Security Research Center, funded, directed and managed this work through Contract Number EP-C-10-001 with Battelle. This report has been peer and administratively reviewed and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use of a specific product.

Questions concerning this document should be addressed to:

Joseph Wood
National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Mail Code E343-06
Research Triangle Park, NC 27711
(919) 541-5029
wood.joe@epa.gov

Foreword

Following the events of September 11, 2001, addressing the critical needs related to homeland security became a clear requirement with respect to EPA's mission to protect human health and the environment. Presidential Directives further emphasized EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological (CBR) attack. To support EPA's mission to assist in and lead response and recovery activities associated with CBR incidents of national significance, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of the Agency and other federal, state and local agencies to carry out their homeland security responsibilities.

One goal of NHSRC's research is to provide information on decontamination methods and technologies that can be used in the response and recovery efforts resulting from a CBR release over a wide area. The complexity and heterogeneity of the wide-area decontamination challenge necessitates the understanding of the effectiveness of a range of decontamination options. In addition to effective fumigation approaches, rapidly deployable or readily available surface decontamination approaches have also been recognized as a tool to enhance the capabilities to respond to and recover from such an intentional CBR dispersion.

Through working with ORD's program office partners (EPA's Office of Emergency Management and Office of Chemical Safety and Pollution Prevention) and Regional on-scene coordinators, NHSRC is attempting to understand and develop useful decontamination procedures for wide-area remediation. This report documents the results of a laboratory study designed to better understand the operational aspects and effectiveness of a commercially available technology that can electrochemically generate a hypochlorous acid-based solution (referred to as "anolyte") that could be used to decontaminate materials contaminated with *Bacillus anthracis* spores; data are also presented on the decontamination efficacy for materials contaminated with *Bacillus subtilis* spores.

These results, coupled with additional information in separate NHSRC publications (available at www.epa.gov/nhsrc) can be used to determine whether a particular decontamination technology can be effective in a given scenario. NHSRC has made this publication available to the response community to prepare for and recover from disasters involving chemical and/or biological contamination. This research is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

Jonathan Herrmann, Director
National Homeland Security Research Center

Acknowledgments

Battelle Memorial Institute

Contributions of the following individuals as reviewers of this report are gratefully acknowledged:

United States Environmental Protection Agency (EPA)
Office of Research and Development, National Homeland Security Research Center
Eletha Brady-Roberts
Brian Attwood

United States Environmental Protection Agency (EPA)
Office of Emergency Management, National Decontamination Team
Paul Kudarauskas

Defense Threat Reduction Agency (DTRA)
Bruce Hinds

Contents

Tables

Figures

Abbreviations/Acronyms

γ	gamma
A	amperage/amp(s)
AC	alternating current
ATCC	American Type Culture Collection
B. anthracis	*Bacillus anthracis* (Ames strain)
B. subtilis	*Bacillus subtilis* (ATCC 19659)
BBRC	Battelle Biomedical Research Center
BSC III	biological safety cabinet, Class III
C	Celsius
CGB	compact glovebox
CFU	colony-forming unit(s)
CI	confidence interval
Cl^-	chloride ion
Cl_2	chlorine gas
ClO^-	hypochlorite ion
cm	centimeter(s)
COR	Contracting Officer's Representative
CPU	central processing unit
D	depth
DC	direct current
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
F	Fahrenheit
FAC	free available chlorine
g	gram(s)
gal	gallon(s)
gpd	gallons per day
gph	gallons per hour
H	height
H^+	hydrogen ion
HCl	hydrochloric acid
HDPE	high density polyethylene
HMI	human machine interface
HOCl	hypochlorous acid
hr	hour(s)
i.d.	internal dimensions
IET	Integrated Environmental Technologies, Ltd.
IP	internet protocol
L	liter(s)
lb	pound(s)
Lph	liters per hour
min	minute
mL	milliliter(s)
mS	millisiemen(s)
mV	millivolt(s)

µL	microliter(s)
NA	not applicable
Na^+	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
NEMA	National Electrical Manufacturers Association
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OCl^-	hypochlorite
ORD	EPA Office of Research and Development
ORP	oxidation-reduction potential
PBS	phosphate-buffered saline
pc	positive control
ppm	part(s) per million
psi	pound(s) per square inch
QA	quality assurance
QAPP	Quality Assurance Project Plan
QC	quality control
QMP	quality management plan
RH	relative humidity
rpm	revolution(s) per minute
SD	standard deviation
SE	standard error
SFW	sterile filtered water (cell-culture grade)
STS	sodium thiosulfate
T&E	Testing and Evaluation
TDS	total dissolved solids
TSA	technical systems audit
V	voltage/volts
W	width
WA	Work Assignment

Executive Summary

The U.S. Environmental Protection Agency's (EPA's) National Homeland Security Research Center (NHSRC) is helping to protect human health and the environment from adverse impacts resulting from the release of threat agents by identifying methods and technologies that can be used for decontamination following an attack using chemical or biological agents. In the study described in this report, an electrochemically-generated hypochlorous acid solution from a modified commercially-available EcaFlo® system was evaluated with regard to its ability to decontaminate six materials inoculated with approximately 1 x 10^8 total colony forming units (CFU) of *Bacillus anthracis* (Ames) and *Bacillus subtilis* spores (ATCC 19659). The materials were typical of surfaces found inside residential buildings.

Porous materials:

- Industrial carpet
- Painted wallboard paper
- Bare pine wood

Non-porous materials:

- Decorative laminate
- Galvanized metal
- Glass

Experimental Procedures. All the material coupons were approximately 1.9 centimeters (cm) by 7.5 cm in size. For testing, coupons were "contaminated" by spiking (i.e., inoculating) with spores of the biological agent, either *Bacillus anthracis* or *Bacillus subtilis*.

The hypochlorous acid-based decontaminant (referred to hereafter as "anolyte") was generated using the EcaFlo® system, according to the vendor's instructions and training. Once generated, the anolyte was transferred into a commercially-available, 500 milliliter (mL) spray bottle to apply the decontaminant from a measured distance until the surfaces of the coupons were fully wetted. Spray distance, humidity, and temperature were the same for all applications, and all coupons were horizontally-oriented (i.e., lying flat) for testing.

The following performance characteristics of the EcaFlo® generator and anolyte were evaluated:

- Optimization of the EcaFlo® generator, to produce anolyte solutions having free available chlorine (FAC) levels of 1000 to 3500 parts per million (ppm) at pH levels of 5, 6, and 7 for each FAC level.
- Stability (useful life) of the anolyte solutions.
  - Determine change in FAC and pH level over time as a function of various FAC and pH levels.
- Decontamination efficacy.
  - Quantitative assessment of the decontamination efficacy for viable organisms (log reduction) for anolyte solutions as a function of various FAC and pH levels.
- Qualitative assessment of damage to material surfaces following decontamination.

Results. Optimization tests were conducted to determine how varying the modified EcaFlo® system flow rate, power input, and salt concentration would affect the FAC and pH levels of the anolyte solutions generated. (Oxidation-reduction potential [ORP] was also measured as another parameter to characterize the anolyte solutions.) The evaluation demonstrated success in achieving the targeted FAC levels of 1,000, 2,000, and 3,000 ppm, each at pH levels of 5, 6, and 7, constituting nine optimization tests. The final optimization test was successful in achieving 3,500 ppm FAC at pH 5.

Due to the reactivity (or volatility) of the anolyte, the concentration of FAC in the solution was expected to diminish somewhat over time. The levels of FAC, pH, and ORP for each anolyte solution were therefore measured immediately following generation. After 1 hour and after 2 hours had passed, these values were measured again. These useful-life evaluations for each anolyte showed that only gradual degradation occurred over the two-hour span for all anolyte solutions, with the exception of one test solution.

With regard to decontamination efficacy, the anolyte was most effective in inactivating *B. anthracis* and *B. subtilis* spores on the non-porous materials (decorative laminate, galvanized metal, and glass), with over 86% of tests resulting in a log reduction ≥ 6. In numerous tests with the non-porous materials, the anolyte achieved complete inactivation (no spores detected), particularly with *B. subtilis*. See Tables E-1 and E-2 for a summary of the decontamination results. The porous materials (industrial carpet, painted wallboard paper, and bare pine wood) were more difficult to decontaminate, with all log reduction results ≤ 3.47.

No visible damage was observed on any of the coupon materials following each of the decontamination conditions tested in this study.

Table E-1. Summary of mean quantitative efficacy (log reduction) results for *Bacillus anthracis* (Ames)

Test Material	Quantitative Efficacy (mean log reduction ± 95% confidence interval)					
	3000 ppm FAC, pH 5, 60 min contact time, two total spray applications	3000 ppm FAC, pH 6, 60 min contact time, two total spray applications	3000 ppm FAC, pH 7, 60 min contact time, two total spray applications	3500 ppm FAC, pH 5, 60 min contact time, two total spray applications	3500 ppm FAC, pH 5, 120 min contact time, four total spray applications	3500 ppm FAC, pH 5,120 min contact time, four total spray applications (18 hr contact)
Industrial Carpet	0.58 ± 0.24	0.26 ± 0.08	0.31 ± 0.06	0.30 ± 0.08	0.60 ± 0.18	0.45 ± 0.17
Decorative Laminate	5.95 ± 1.05	7.55 ± 0.23[a]	7.28 ± 0.74[a]	4.88 ± 2.05	7.50 ± 0.23[a]	7.60 ± 0.05[a]
Galvanized Metal	4.58 ± 0.12	7.46 ± 0.72	7.61 ± 0.60	7.60 ± 0.60	7.81 ± 0.04[a]	7.68 ± 0.10[a]
Painted Wallboard Paper	2.57 ± 0.18	2.18 ± 0.30	2.62 ± 0.64	2.43 ± 0.45	2.37 ± 0.70	3.47 ± 0.26
Bare Pine Wood	2.13 ± 0.26	0.68 ± 0.19	1.02 ± 0.23	0.81 ± 0.15	0.89 ± 0.37	0.97 ± 0.42
Glass	4.55 ± 0.14	7.83 ± 0.07[a]	7.93 ± 0.04[a]	7.62 ± 0.60[a]	7.87 ± 0.02[a]	7.73 ± 0.13[a]

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/material.

Table E-2. Summary of mean quantitative efficacy (log reduction) results for *Bacillus subtilis* (ATCC 19659)

Test Material	Quantitative Efficacy (mean log reduction ± 95% confidence interval)					
	3000 ppm FAC, pH 5, 60 min contact time, two total spray applications	3000 ppm FAC, pH 6, 60 min contact time, two total spray applications	3000 ppm FAC, pH 7, 60 min contact time, two total spray applications	3500 ppm FAC, pH 5, 60 min contact time, two total spray applications	3500 ppm FAC, pH 5, 120 min contact time, four total spray applications	3500 ppm FAC, pH 5,120 min contact time, four total spray applications (18 hr contact)
Industrial Carpet	0.73 ± 0.07	0.21 ± 0.06	0.61 ± 0.13	0.65 ± 0.12	0.97 ± 0.09	0.84 ± 0.09
Decorative Laminate	5.95 ± 0.88	7.57 ± 0.06[a]	6.91 ± 0.16[a]	6.12 ± 0.86	7.62 ± 0.02[a]	7.54 ± 0.07[a]
Galvanized Metal	7.71 ± 0.07[a]	7.79 ± 0.04[a]	7.98 ± 0.05[a]	7.06 ± 0.87	7.60 ± 0.05[a]	7.60 ± 0.04[a]
Painted Wallboard Paper	1.71 ± 0.65	2.51 ± 0.25	3.01 ± 0.08	2.56 ± 0.47	2.44 ± 0.55	3.45 ± 0.64
Bare Pine Wood	0.30 ± 0.14	0.47 ± 0.09	0.67 ± 0.25	0.54 ± 0.43	0.76 ± 0.50	0.67 ± 0.39
Glass	6.14 ± 1.05	7.75 ± 0.04[a]	7.85 ± 0.11[a]	7.71 ± 0.19[a]	7.66 ± 0.08[a]	7.60 ± 0.04[a]

[a]Result represents complete inactivation within the detectable limit of 33.33 CFU/material.

1.0 Introduction

The U.S. Environmental Protection Agency's (EPA's) National Homeland Security Research Center (NHSRC) is helping protect human health and the environment from adverse impacts resulting from the release of chemical, biological, or radiological agents. With an emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, NHSRC is working to develop tools and information that will help detect the intentional introduction of chemical or biological contaminants in buildings or water systems, contain these contaminants, decontaminate buildings or water systems, and facilitate the disposal of material resulting from remediation activities.

NHSRC works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the participation of individual technology developers in carrying out performance tests on homeland security technologies. The program evaluates the performance of innovative homeland security technologies by developing test plans that are responsive to the needs of stakeholders, conducting tests, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) project plans (QAPPs) to ensure that data of known and high quality are generated and that the results are defensible. This program provides high-quality information that is useful to decision makers in purchasing or applying the tested technologies and provides potential users with unbiased, third-party information that can supplement vendor-provided information. Stakeholder involvement ensures that user needs and perspectives are incorporated into the test design so that useful performance information is produced for each of the tested technologies.

In this study, the efficacy of a hypochlorous acid decontaminant solution (anolyte) generated by an electrochemical process in inactivating *Bacillus anthracis* (Ames) and *B. subtilis* (American Type Culture Collection (ATCC) 19659) spores on common building materials was evaluated. Both porous (industrial carpet, painted wallboard paper, and bare pine wood) and non-porous (decorative laminate, galvanized metal ductwork, and glass) materials were used in this evaluation. Anolyte solutions at a free available chlorine (FAC) concentration of 3,000 parts per million (ppm) at pH levels of 5, 6, and 7, and at an FAC concentration of 3,500 ppm at pH 5 were used in the decontamination efficacy tests. Decontamination efficacy was determined based on the log reduction in viable spores recovered from the inoculated materials (with and without exposure to the anolyte).

Ten "optimization" tests were also conducted to evaluate how decreasing the EcaFlo® flow rate, increasing the power input, increasing the salt concentration, and/or other parameters determined or recommended by the vendor affected the levels of pH, oxidation-reduction potential (ORP), and FAC in the anolyte solutions

generated. These tests were conducted based on recommendations provided by the vendor to achieve target FAC levels of 1,000, 2,000, and 3,000 ppm, each at pH levels of 5, 6, and 7.

Due to the reactivity (and volatility) of the anolyte solution, the concentration of FAC in the solution was expected to degrade somewhat over time. (Vendor literature indicated that loss of FAC could occur at high temperature and with exposure to direct sunlight.) Therefore, in the useful life tests, the level of FAC, ORP, and pH for each decontaminant solution was measured immediately following generation. After 1 hour and after 2 hours had passed, these values were measured again.

2.0 Technology Description

The primary purpose of this evaluation was to investigate the use of electrochemically-generated anolyte solutions produced from the commercially-available EcaFlo® Model No. C-102 system (Figure 2-1, Table 2-1, Integrated Environmental Technologies, Ltd. [IET], Little River, SC) to decontaminate building or outdoor materials that have been contaminated with *B. anthracis* (Ames) and *B. subtilis* (ATCC 19659) spores. Normal EcaFlo® system settings permit the generation of anolyte solution comprised of 0.03% to 0.05% (350 to 500 ppm) hypochlorous acid (HOCl). For this evaluation, however, IET furnished a modified EcaFlo® system (at our request) that exceeded normal function to meet testing demands (Table 2-2). The EcaFlo® system was modified to produce anolyte with higher FAC and lower pH levels to improve sporicidal efficacy. The EcaFlo® system Model No. C-102 is typically supplied with two electrolytic cells that are operated in parallel to each other, but for this evaluation, the modified model had the two electrolytic cells operating in series to allow the solution to make two passes instead of a single pass to achieve better salt conversion and hence solutions with higher FAC concentration. (We do note that this modified configuration is now commercially available.) In addition to the two electrolytic cells configured in series, the operation of the unit was modified (by adjusting brine pump speed, electrical current, and flow rate) to produce higher FAC concentrations and lower pH levels, again to optimize the unit for improved sporicidal efficacy. The values in Table 2-2 were provided by IET to indicate the anolyte solution characteristics produced by the different EcaFlo® system settings achieved at IET's facility using treated water from Little River, SC (this information was not verified as part of this evaluation), for the 2-cell in parallel and 2-cell in series configurations. These values were expected to change somewhat since the present evaluation was performed using the water from the test facility located at West Jefferson, OH, and water quality and temperature could potentially affect performance.

Figure 2-1. Front and side views of EcaFlo® Model C-102 (modified version)

Table 2-1. Specifications and Performance Standards for EcaFlo® Model C-102 (unmodified)

Specifications	
Overall cabinet dimensions	36” width (W) x 25” depth (D) x 65” height (H)
Weight (Dry)	340 lbs (154 kg)
Cabinet enclosure	Stainless steel, NEMA 4X
Portability	Block- or locking-wheel mounted
Power Requirements	220 voltage (V) Alternating Current (AC), dedicated 30 amperage (A) circuit, clean reliable power with a surge suppressor that also mitigates in-house generated transients
Water source	Minimum 35 pounds per square inch (psi) with ¾” internal dimension (i.d.) service line delivering a minimum 50 gallons per hour (gph), softened to < 1 grain per gallon hardness total dissolved solids (TDS), < 5 micron sediment size
Controller	Touch screen human machine interface (HMI) interface with central processing unit (CPU) for semi-automatic operation, remote operation with Ethernet/internet protocol (IP) connection
Brine tank	35 gal external with circulation pump
Operating temperatures	Ambient air in which to operate equipment is between 10 °C (50 °F) and 27 °C (80 °F)
Performance Standards	
Production capacity	756 gallons (gal) of solutions per day 33.6 gal anolyte per hour, 605 gallons per day (gpd) 8.4 gal catholyte per hour, 151 gpd
FAC concentrations	350 to 500 ppm
Current consumption	100A Direct Current (DC)
Brine concentration	3 to 7 grams (g) per liter (L)
Production run times	18 hours max per 24 hour period 4.5 hours max continuous duty cycle, followed by a flush and 1.5 hr “rest” period

Table 2-2. Operational parameters for normal 2-cell in parallel configuration of EcaFlo® Model C-102 vs. the 2-cell in series, modified configuration, to achieve targeted FAC and pH levels

Target values (ppm, pH)	Actual FAC[1] (ppm)[2]	pH[2]	Brine Pump (%)[3]	Amps (A) Set/Actual[3]	Flow (gph)[2]	ORP (mV)[2]
2-Cell in Parallel Configuration						
800, 5	819	5.05	45	50/NA[4]	42	1115
800, 6	835	6.03	50	50/NA	42	1050
800, 7	795	7.00	50	50/NA	40	1005
1000, 5	1028	5.10	40	45/NA	34	1104
1000, 6	1020	6.03	40	45/NA	33	1022
1000, 7	1022	7.06	40	45/NA	30	960
1200+, 5	1752	5.03	50	50/NA	28	1125
1200+, 6	1710	5.99	50	50/NA	28	1037
1200+, 7	1330	7.03	50	50/NA	28	975
2-Cell in Series Configuration (Modified for testing)						
1000, 5	1040	5.02	20	45/88.8	22	1055
1000, 6	1120	6.03	20	45/88.9	22	977
1000, 7	916	7.05	20	45/88.3	22	922
2000, 5	2030	5.15	30	45/91.5	20	1090
2000, 6	1985	5.95	30	45/91.5	20	1025
2000, 7	2250	7.06	35	50/102.9	18	949
3000, 5	3400	4.98	50	60/104.2	16	1113
3000, 6	3300	5.96	50	60/104.3	16	1047
3000, 7	2794	6.97	50	60/104.4	16	975

[1]FAC represents the chlorine available for decontamination, primarily in the form of HOCl (depending on pH).

[2]measured values associated with anolyte generation/production.

[3]EcaFlo® system settings.

[4]Not applicable.

The decontamination efficiency of chlorine or chlorine-containing compounds is dependent on the chemical form. In aqueous solutions, chlorine can be in many forms:

- Chloride ion (Cl^-)
- Dissolved gas – chlorine gas (Cl_2)
- Inorganic oxyanion (ClO^- or OCl^-, hypochlorite ion)
- Inorganic complex (HOCl, hypochlorous acid).

Of these forms, HOCl is the strongest oxidizer. Hypochlorous acid is a highly reactive, unstable, weak acid that quickly degrades once formed ($HOCl \rightleftharpoons OCl^-$ + hydrogen ion [H^+]) and has been used as a sporicide and disinfectant in numerous applications. There are several ways to produce HOCl, but the EcaFlo® system produces HOCl by electrolysis or what IET calls "Electro-Chemical Activation."[1] A weak brine solution (using sodium chloride, or NaCl) was passed through a series of two electrolytic cells of Model C-102 for this evaluation. The weak brine is created when the brine pump meters a small amount of concentrated brine into the supply water as it flows through the device. Current applied across the titanium electrodes and through an ion-permeable ceramic separator within the electrolytic cells causes the NaCl to dissociate, thereby moving the negatively charged chloride ions (Cl^- anions) from the salt to flow to the anode (+). The positively charged sodium ions (Na^+ cations) from the dissociated salt flow to the cathode (-). Therefore, the "anolyte" solution contains the HOCl formed in the anode of the electrolytic cells, and the "catholyte" solution contains sodium hydroxide (NaOH) formed at the cathode of the electrolytic cells. Typical anolyte has a pH of 6.5 to 7.5, and the catholyte has a pH of 12.0, and can be used as a mild degreaser.[1]

When the configuration of the two electrolytic cells is in series, the brine initially flows through the cathode chamber of the first cell. A very small portion of catholyte and hydrogen gas is removed from this solution before all of it goes through the anode chamber of this first cell. Upon exiting the anode chamber of the first cell, the anolyte goes through the cathode chamber of the second cell and 20 to 35% (a minimum of 200 mL and a maximum of 350 mL catholyte for every liter of anolyte generated) of the solution leaves this cell as catholyte.

A critical factor that determines the level of HOCl in water is pH. A higher pH allows the formation of more OCl^- and results in less HOCl. Therefore, disinfection is theoretically more effective at a lower pH, which favors the presence of HOCl in the water over the other forms. The regulation of catholyte regulates the pH of the final anolyte product. As more of the higher pH catholyte is forced into the anode chamber, the pH of the anolyte becomes higher. Similarly, if the amount of high pH catholyte is decreased in the anode chamber by opening the throttle needle valve (Figure 2-2), the pH of the final anolyte product will decrease. During this evaluation, however, the throttle was a secondary method of adjusting the pH. The primary method as recommended by IET (private communication during tests) was to raise or lower the catholyte solution line (Figure 2-3) manually, to decrease or increase, respectively, the removal of the high pH catholyte solution from the unit. The anolyte and catholyte solutions are concurrently generated by the EcaFlo® system, and both solutions exit the EcaFlo® system through ports and tubing (i.e., lines) into a waste container or collection container. Since the anolyte was the solution of interest for this evaluation, the line for this solution was diverted at

intervals into a collection container. The catholyte line, however, was raised to increase the pH of the anolyte by forcing the high-pH catholyte to remain in the electrolytic cells for longer periods. The catholyte line was raised to a height of 198.12 cm (78 inches) when the higher FAC anolyte (2,000 ppm, 3,000 ppm, and 3,500 ppm) was generated. For the 1,000 ppm FAC anolyte, the catholyte line was lowered to 101.60 cm (40 inches). Both adjustments were recommended by IET.

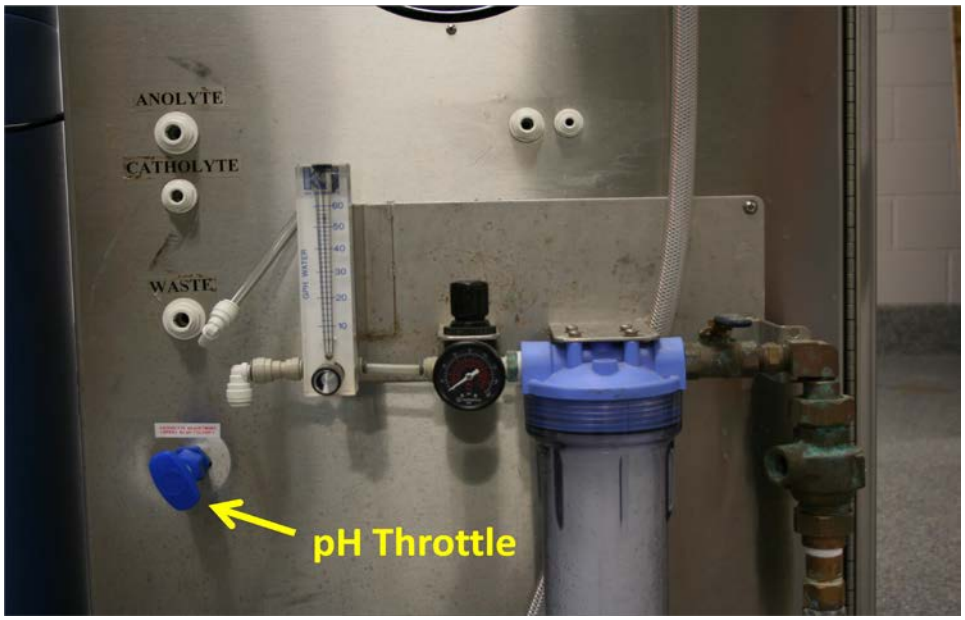


Figure 2-2. EcaFlo® system throttle

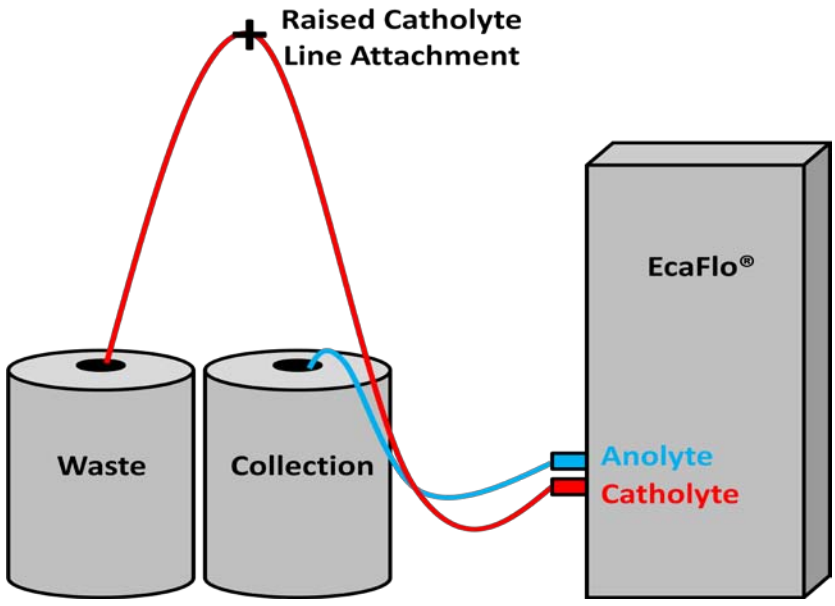


Figure 2-3. Diagrammatic illustration showing raised catholyte line

A calibrated pH meter (Cole-Parmer Item No. BU-35615-20, Vernon Hills, IL) was submerged in the collection container as the anolyte was generated and gave real-time readings while the solution was stirred. If the pH was not within the target range, the collected anolyte was immediately transferred into the waste container and the catholyte line was adjusted, followed by more pH readings. If adjustment of the catholyte line failed to achieve the target pH, the throttle was adjusted to augment the pH. A minimum of 1 L was required for testing. A minimum of 500 mL was needed to perform the spray testing (i.e., application of decontaminant with spray bottle to *B. anthracis*- or *B. subtilis*-inoculated surfaces), and a minimum of 500 mL anolyte was required to perform useful-life testing (i.e., evaluation of FAC and pH of decontaminant over the course of two hours from the time of generation). Newly-generated anolyte with the proper pH was then immediately titrated using an iodometric method to determine the FAC concentration (ppm). An iodometric method (Total Chlorine Test Kit , Model No. CN-DT, Hach Company, Loveland, CO) and a digital titrator (Hach Model 16900), as specified by IET, were used to measure FAC concentration in each anolyte solution generated. With the anolyte solutions, total chlorine is equal to FAC. These equipment items were combined as one unit (Hach Item No. 2471100). Manufacturer procedures provided with the titrator were followed for this measurement; the kit's chlorine standards were used to verify accuracy.

The composition of the brine solution was salt-saturated, softened tap water. The unheated tap water was from a municipal source (West Jefferson, OH) that had to be softened to a measured hardness of < 1 ppm, as prescribed by IET[1], since minerals present in the water would cause scale build-up inside the electrolytic cells, reducing function. In this study, the EcaFlo system was de-scaled once per week to improve anolyte generation efficiency as recommended by IET since high, anolyte FAC concentrations were targeted and achieved. The brine solution was prepared using an IET-specified, commercially-available softener salt (Diamond Crystal® Pellets with Softener Care™, Cargill Item No. 7336/7337, Minneapolis, MN) from a home improvement retailer, and the salt was allowed to saturate the water in the continuously circulating brine tank (also provided by IET) for a minimum of 24 hours prior to anolyte generation. A saturated salt solution ensures the production of anolyte using consistent EcaFlo® settings specific for the targeted concentration, whereas, partially dissolved salt would require adjustments depending on the concentration of the salt solution attained at the time of EcaFlo® operation. IET stated in the operator's manual for the EcaFlo® Model C-102 system that other salt manufacturers may provide similar products, but the Cargill product was chosen because of the salt's purity (99.8%), dissolving rate, and availability to IET from local suppliers.

The anolyte (an oxidizing chemical) is able to gain or acquire electrons from the cell membranes of the microorganisms in solution. The loss of electrons from the cell membranes acts to destabilize the membranes, reduce their integrity, and results in the eventual inactivation of the microorganisms affected. Therefore, the ORP was measured as a secondary indicator of the antimicrobial or disinfection capability of the anolyte. The ORP measurement was performed using an ORP probe to measure the voltage (in millivolts) of the anolyte generated; refer to Section 3.5 for further details. The brine pump speed, amperage, and flow were other process parameters measured, and these were displayed on the EcaFlo®'s human machine interface (HMI).

3.0 Summary of Test Procedures

Test procedures were performed in accordance with the peer-reviewed QAPP and are briefly summarized in this chapter.

3.1 Preparation of Test Coupons

The *B. anthracis* spores used for this testing were prepared from a qualified stock of the Ames strain at the Battelle Biomedical Research Center (BBRC). *Bacillus anthracis* spore lots were subject to a stringent characterization and qualification process, required by Battelle's standard operating procedure for spore production. Specifically, *B. anthracis* spore lots were characterized prior to use by observation of colony morphology, direct microscopic observation of spore morphology and size, and determination of percent refractivity and percent encapsulation. In addition, the number of viable spores was determined by colony count and expressed as colony forming units per milliliter (CFU/mL). Theoretically, once plated onto bacterial growth media, each viable spore germinates and yields one CFU. Variations in the expected colony phenotypes were recorded. Endotoxin concentration of each spore preparation was determined by the Limulus Amebocyte Lysate assay. Genomic deoxyribonucleic acid (DNA) was extracted from the spores and DNA fingerprinting was done to confirm the genotype. The virulence of the *B. anthracis* spore lot was measured by challenging guinea pigs intradermally with a dilution series of spore suspensions, and virulence was expressed as the intradermal median lethal dose. In addition, testing was conducted for robustness of the spores via hydrochloric acid (HCl) resistance. The *B. anthracis* stock spore suspension was prepared in sterile, filtered water at an approximate concentration of 1×10^9 spores/mL and stored under refrigeration at 2 to 8 °C.

B. anthracis or *B. subtilis* spores were inoculated onto test coupons in an appropriate biosafety cabinet (BSC III) according to established BBRC procedures. Inoculated coupons were prepared prior to each day of experimental work. Coupons were placed flat in the BSC III and inoculated at approximately 1×10^8 total spores per coupon. This inoculation was accomplished by dispensing a 100 microliter (µL) aliquot of the spore stock suspension (approximately 1×10^9 spores/mL) using a multi-channeled micropipette as 10 droplets (each of 10 µL volume, Figure 3-1) across the surface of the test coupon. This approach provided more uniform distribution of spores across the coupon surface than would be obtained through a single drop of the suspension. After inoculation, the test coupons remained undisturbed overnight in the BSC III to dry thoroughly. Test coupons were then exposed to anolyte the next day (i.e., within 24 hours after inoculation).

The origin and specifications of the materials used for test coupons are shown in Table 3-1. All materials were selected as representative types of building materials. All test coupons were made from new materials. Test coupons were 1.9 x 7.5 centimeters (cm) in size.

Coupons were sterilized before use by gamma (γ)-irradiation (industrial carpet, painted wallboard paper, bare pine wood, and decorative laminate) or autoclaving (galvanized metal and glass). The γ-irradiation sterilization method was chosen for the porous materials since the pressure (15 psi) and heat (121 °C) from an autoclave could physically alter or damage these coupons. Therefore, the porous coupons were sent to be γ-irradiated at approximately 40 kilogray by a vendor that specializes in this type of processing (STERIS Isomedix Services, Libertyville, IL). The non-porous materials were autoclaved at Battelle by following an internal standard operating procedure.

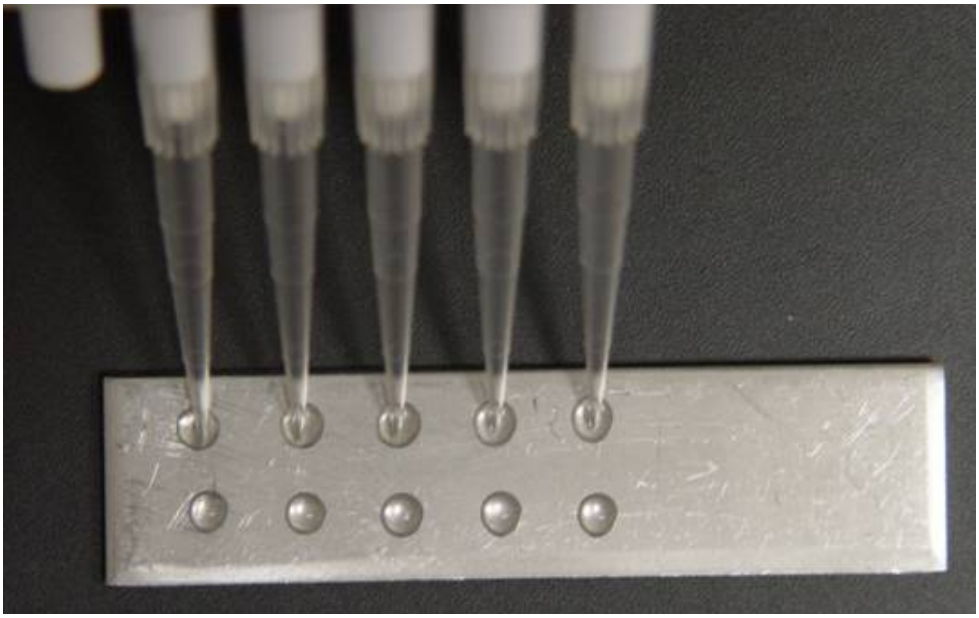


Figure 3-1. Inoculation of coupon using a multi-channeled micropipette

Table 3-1. Summary of materials used for decontaminant testing

Material	Lot/Batch/ Observation	Manufacturer/ Supplier Name	Coupon Size, Width x Length	Material Preparation
NON-POROUS				
Glass	C1036	Brooks Brothers Glass; Columbus, OH	1.9 cm x 7.5 cm	Autoclave
Galvanized metal ductwork	NA[1]	Adept Products; West Jefferson, OH	1.9 cm x 7.5 cm	Autoclave
Decorative laminate	NA	A'Jack Inc.; Columbus, OH	1.9 cm x 7.5 cm	γ- irradiation
POROUS				
Carpet	Shaw EcoTek 6	Grossmans Bargain Outlet; Columbus, OH	1.9 cm x 7.5 cm	γ- irradiation
Painted wallboard paper	05-16-03; Set-E-493; Roll-3	United States Gypsum Company; Chicago, IL	1.9 cm x 7.5 cm	γ- irradiation
Bare pine wood	Generic modeling	West Jefferson Hardware, West Jefferson, OH	1.9 cm x 7.5 cm	γ- irradiation

[1]Not applicable.

3.2 Decontaminant Testing

Five replicate test coupons (inoculated with *B. anthracis* or *B. subtilis* spores and decontaminated), five replicate positive control coupons (inoculated and not decontaminated), one procedural blank (not inoculated, decontaminated), and one laboratory blank (not inoculated, not decontaminated) of each coupon material were used in testing with each batch of anolyte generated under a different EcaFlo® system configuration. In testing of each anolyte, all test coupons were oriented horizontally (i.e., lying flat). Anolyte runoff and anolyte pooled on top of each test coupon were captured, neutralized, and subjected to spore extraction along with the associated test coupon.

On the day following inoculation, test coupons intended for decontamination (including blanks) were separated from the positive controls or coupons not exposed to decontaminant (including blanks) since both sets were inoculated and dried overnight in the same BSC III. Prior to the start of spray testing, laboratory resources were coordinated to quickly receive the fresh anolyte solution generated from the EcaFlo® system. Once both pH and FAC targets were achieved for that particular anolyte batch, the anolyte was immediately transferred into a commercially-available, high density polyethylene (HDPE) container as specified by IET and filled to leave as little head-space in the container as possible to mitigate off-gassing. This HDPE container was sealed and immediately transported into the laboratory to conduct spray testing. Inside the laboratory, the anolyte was transferred into a commercially-available, 16 ounce (480 mL) sprayer with a cylinder style, HDPE bottle (Qorpak® Item No. 7331X, Bridgeville, PA). No more than five minutes elapsed from the time of FAC measurement (the final measurement of the freshly-generated anolyte solution) to the start of decontamination testing. The anolyte spray distance (30.5 cm), humidity ($\leq$ 70% relative humidity (RH)), and

temperature (22 °C ± 2 °C) were the same for all applications, including the positive controls.

The quantity of anolyte sprays (i.e., spray application number) varied for each testing parameter (e.g., two- or four-spray applications). Whenever an application of anolyte was needed, each material warranted a specific number of sprays (i.e., pulls of the sprayer trigger) to ensure that the surfaces were fully wetted with anolyte. For instance, during one evaluation, two spray applications of anolyte were required with a 60 minute total contact time. Each application (i.e., Time 0, +30) required four trigger pulls of the sprayer to wet treated wood fully. Only three trigger pulls were needed to wet glass fully. The reason that an additional trigger pull was needed to fully wet the treated wood was due to it being a porous material. Porous materials absorbed the anolyte, so more volume was needed until it was determined that the treated wood (in this case) was fully wet with anolyte. This was also the case with each re-application. The positive controls (including blanks) were transferred into a separate glovebox (Compact Glove Box, Plas-Labs Model No. 830-ABC, Lansing, MI) where sterile filtered water (SFW) was sprayed using the same type of sprayer.

Following decontamination, each coupon (along with any associated runoff and pooled decontaminant) was aseptically transferred to a sterile 50 mL conical tube containing 10 mL of sterile phosphate-buffered saline (PBS) solution with 0.1% Triton X-100 surfactant (i.e., 99.9% PBS, 0.1% Triton X-100) and the appropriate concentration of sodium thiosulfate (STS) neutralizer needed to stop the decontamination activity of the anolyte. The required concentration of STS was determined in the neutralization panels for each anolyte or number of applications tested. In each of the neutralization panels, a range of STS concentrations was tested to determine the concentration that most effectively stopped the action of the anolyte (as indicated by the maximum recovery of viable spores in simulated coupon extracts). The results of those trial runs are shown in the respective decontamination results chapters (Chapters 6 to 11).

After the coupons were transferred to the conical tubes, spores were extracted from the coupons by agitation on an orbital shaker for 15 minutes at approximately 200 revolutions per minute (rpm) at room temperature. Following extraction, a 1 mL aliquot of the coupon extract was removed, and a series of dilutions up through 10^{-7} was prepared in SFW. An aliquot (0.1 mL) of the undiluted extract and each serial dilution were then spread-plated onto tryptic soy agar plates (in triplicate) and incubated overnight at 35 to 37 ºC. Resulting colonies were enumerated within 18 to 24 hours of plating. The number of CFU/mL was determined by multiplying the average number of colonies per plate by the reciprocal of the dilution and accounting for the 0.1 mL volume of the extract or dilution that was plated.

Before further decontamination tests with the next anolyte solution, the BSC III and the compact glove box (CGB) were thoroughly cleaned following procedures established under the BBRC Facility Safety Plan.

Laboratory blanks controlled for sterility, and procedural blanks controlled for viable spores inadvertently introduced to test coupons. The procedural blanks were spiked with an equivalent amount of 0.1 mL of “stock suspension” that did not contain the biological agent. The target acceptance criterion was that extracts of laboratory or procedural blanks were to contain no CFU. The mean percent spore recovery from each coupon material was calculated using results

from positive control coupons (spiked, not decontaminated; sprayed with SFW instead of the decontaminant), by means of the following equation:

$$\text{Mean \% Recovery} = [\text{Mean } CFU_{pc}/CFU_{spike}] \times 100 \quad (1)$$

where Mean CFU_{pc} is the mean number of CFU recovered from five replicate positive control coupons of a single material, and CFU_{spike} is the number of CFU spiked onto each of those coupons. The value of CFU_{spike} is known from enumeration of the stock spore suspension. Spore recovery was calculated for *B. anthracis* or *B. subtilis* on each coupon material, and the results are included in Chapters 6 through 11.

3.3 Decontamination Efficacy

The performance or efficacy of the anolyte was assessed by determining the number of viable organisms remaining on each test coupon and in any decontaminant run-off from the coupon after decontamination. Those numbers were compared to the number of viable organisms extracted from the positive control coupons, which were sprayed with SFW instead of with the anolyte.

The number of viable spores of *B. anthracis* or *B. subtilis* in extracts of test and positive control coupons was determined to calculate efficacy of the decontaminant. Efficacy is defined as the extent (as $\log_{10}$ reduction) to which viable spores extracted from test coupons after decontamination were less numerous than the viable spores extracted from positive control coupons subjected only to an inert aqueous spray, at the same temperature and contact time as the decontaminant application. First, the logarithm of the CFU abundance from each coupon extract was determined, and then the mean of those logarithm values was determined for each set of control and associated test coupons, respectively. Efficacy of a decontaminant for a test organism on the i^{th} coupon material was calculated as the difference between those mean log values, i.e.:

$$Efficacy = \overline{(\log_{10} CFUc_{ij})} - \overline{(\log_{10} CFUt_{ij})} \quad (2)$$

where $\log_{10} CFUc_{ij}$ refers to the j individual logarithm values obtained from the positive control coupons and $\log_{10} CFUt_{ij}$ refers to the j individual logarithm values obtained from the corresponding test coupons, and the overbar designates a mean value. In tests conducted under this plan, there were five control and five corresponding test coupons (i.e., $j = 5$) for each coupon material. In the case where no viable spores were found in any of the five test coupon extracts after decontamination, a CFU abundance of 1 was assigned, resulting in a $\log_{10}$ CFU of zero for that material. Finding no viable spores occurred when an anolyte solution was highly effective, and no viable spores were found on the decontaminated test coupons. In such cases, the final efficacy on that material was reported as greater than or equal to ($\geq$) the value calculated by Equation 2.

The variances (i.e., the square of the standard deviation) of the $\log_{10} CFUc_{ij}$ and $\log_{10} CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., S^2c_{ij} and S^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 2, as follows:

$$SE = \sqrt{\frac{S^2c_{ij}}{5} + \frac{S^2t_{ij}}{5}} \quad (3)$$

where the number 5 again represents the number *j* of coupons in both the control and test data sets. Each efficacy result is thus reported as a log reduction value with an associated SE value. The significance of differences in efficacy across different coupon materials and spore types was assessed based on the 95% confidence interval of each efficacy result. The 95% confidence interval (CI) is:

$$95\%\ \text{CI} = Efficacy \pm (1.96 \times \text{SE}) \quad (4)$$

Differences in efficacy were judged to be significant if the 95% CIs of the two efficacy results did not overlap. The efficacy results are presented in a series of tables in Chapters 6 through 11 for each anolyte by coupon material and presented in Figures 12-1 and 12-2 in the same format.

3.4 Decontaminant Neutralization Trials and Qualitative Assessment of Surface Damage

Neutralization panels were conducted before any testing with each anolyte batch, using coupons that had not been inoculated with spores. In these neutralization panels, the anolyte was applied, and measurements were made with multiple coupons of each material type to determine the amount of the anolyte that deposited (remained on), or ran off from, each material (i.e., "spray and weigh"). These anolyte deposition data were used in the calculation of efficacy on each respective material, and in neutralization panels to determine the amount of neutralizing agent (STS) needed to stop the action of the decontaminant after the prescribed contact time. In addition, visual inspection of each coupon surface took place after the prescribed anolyte contact time and application rates, through side-by-side comparison of the decontaminated test surface and control coupons of the same test material. Differences in color, reflectivity, and roughness were assessed qualitatively, and observations were documented.

3.5 FAC, pH, ORP, and Conductivity Calibration Methods

An iodometric Total Chlorine Test Kit (Model No. CN-DT, Hach Company, Loveland, CO) and a digital titrator (Hach Model 16900), as specified by IET, was used to measure FAC concentration in each anolyte solution generated. With the anolyte solutions, total chlorine is equal to FAC. These equipment items are combined as one unit (Hach Item No. 2471100). Manufacturer procedures provided with the titrator were followed for this measurement. A chlorine standard was used to verify proper function of the titrator.

A pH meter (Cole-Parmer Item No. BU-35615-20, Vernon Hills, IL) was used to measure the pH of each anolyte solution. The pH probe was a potassium chloride-saturated electrode that was calibrated with standard buffer solutions.

An ORP electrode (Cole-Parmer Item No. YO-35805-15) was used to probe the

oxidizing power of each anolyte solution electrochemically. The ORP probe was calibrated with standard pH buffer solutions saturated with quinhydrone crystals. The calibration process was the following:

- STEP 1: The theoretical potential (mV) should be 92±10 mV at 20 °C or 86±10 mV at 25 °C in pH 7.0 calibration buffer saturated with quinhydrone crystals.

- STEP 2: The theoretical potential should be 268±10 mV at 20 °C or 263±10 mV at 25 °C, in pH 4.0 calibration buffer saturated with quinhydrone crystals. The theoretical potential obtained for the pH 7.0 solution in STEP 1 was subtracted from the theoretical potential obtained for the pH 4.0 solution in STEP 2. The difference should be 177±20 mV.

The ORP was measured for the anolyte solutions generated for the optimization tests (Chapter 5) as well as the decontamination tests (Chapters 6 to 11).

The conductivity of each brine solution used to generate anolyte solutions was measured using the Oakton CON 6 meter with probe (Cole-Parmer Item No. EW-35604-24). A calibration standard was used to calibrate the probe and meter.

Temperatures were monitored but no efforts were undertaken to control any of the test temperatures.

3.5 Anolyte Useful-Life Evaluation

On each day of testing, the anolyte was generated from the modified EcaFlo® system using the modified configuration as a basis to make the anolyte solutions listed in Table 2-2. The FAC, ORP, and pH were verified, and conductivity of the brine solution taken and documented. The anolyte was transferred to a commercially-available spray bottle leaving minimal head-space to mitigate off-gassing during the contact time and multiple applications. The anolyte was then sprayed onto the test coupons, and close observation of the respective material surfaces was made to ensure that they were thoroughly wetted (approximately four squeezes of the trigger per material per application were needed to produce wetting across the surfaces of all five replicates and corresponding blank for each material type).

All tests were conducted at ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature, measured to be 22 °C (± 2 °C). The RH inside the test chamber was monitored with a National Institute of Standards and Technology (NIST)-traceable hygrometer. Whenever the RH reached 70% inside the CGB for the positive controls, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 70%. The BSC III did not need a dehumidification system since the volume of this test chamber was large enough to prevent a quick build-up of RH during spray testing. Therefore, the testing chamber was always ≤ 70% RH during the decontamination of test materials with anolyte.

After one hour had elapsed from the moment of anolyte generation, the FAC, ORP, and pH readings were taken of the anolyte stock that was stored in a sealed container with minimal head-space for useful-life evaluation. These measurements were taken again after two hours had elapsed from the moment of anolyte generation. These elapsed times were chosen for testing because they were determined to be representative of the elapsed times these anolyte solutions would most likely be used in the field before their replacement. The results of the anolyte

useful-life determination are shown in the respective results chapters (Chapters 6 to 11).

4.0 Quality Assurance/Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the Quality Management Plan (QMP)[2] and the QAPP. The QA/QC procedures are summarized below.

4.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, biological safety cabinets, pH meter, ORP probe, conductivity meter, digital titrator, EcaFlo® system) and monitoring devices (e.g., temperature of area where anolyte was generated and testing chamber, RH of testing chamber) used at the time of evaluation were verified as being certified, calibrated, or validated within the valid timeframe of use.

4.2 QC Results

Quality control efforts conducted during decontaminant testing included positive control coupons (inoculated, not decontaminated), procedural blanks (not inoculated, decontaminated), laboratory blanks (not inoculated, not decontaminated), and spike control samples (analysis of the stock spore suspension). The results for these QC samples in each decontaminant evaluation are included in the results chapter for each respective anolyte generated (i.e., see Chapters 6 to 11).

4.3 Audits

4.3.1 Performance Evaluation Audit

Performance evaluation audits were conducted to assess the quality of the results obtained during evaluation.

No performance evaluation audits were performed to confirm the concentration and purity of *B. anthracis* or *B. subtilis* spores because quantitative standards do not exist for these organisms. The control coupons and blanks support the spore measurements.

Table 4-1 summarizes the performance evaluation audits that were performed.

Table 4-1. Performance evaluation audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Volume of liquid from micropipettes	Gravimetric evaluation	± 10%	± 5%
FAC	Compare to chlorine standard	80-120% recovery	90% ± 5%
pH, ORP	Compare with standard solutions	± 0.1 pH unit, ± 20 mV	± 0.1 pH unit, ± 10 mV
Conductivity	Compare to standard solution	± 0.5%	± 0.5%
Temperature	Compared to independent calibrated thermometer	± 2 °C	± 2 °C
Time	Compare time to independent clock or watch value	± 2 sec/hr	0 second/hr

4.3.2 Technical Systems Audit

Battelle QA staff conducted a technical systems audit (TSA) on March 4, 2011, to ensure that the tests were being conducted in accordance with the appropriate QAPP and QMP[1]. As part of the audit, test procedures were compared to those specified in the QAPP and data acquisition and handling procedures were reviewed. Observations and findings from the TSA were documented and submitted to the Battelle work assignment (WA) Leader for response. Two deviations were noted during the audit, but none of the findings of the TSA required corrective action. TSA records were permanently stored with the Battelle QA Manager.

EPA QA staff also conducted a TSA on March 9 through 11, 2011. EPA QA staff reviewed plate counting, spray and weigh activities, and a neutralization panel. Observations and findings from the TSA were documented and submitted to the EPA Contract Officer Representative and Battelle QA Manager for response. The EPA TSA report had six findings, of which only three required any corrective actions. A comment response document was provided to the EPA on April 14, 2011, with all corrective actions documented. A copy of this response document was permanently stored with the Battelle QA Manager.

4.3.3 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. A Battelle QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.4 QAPP Amendments and Deviations

Five deviations were prepared, approved, and retained in the test files for this evaluation. One deviation related to the initiation of testing as described in the QAPP without a fully-signed/approved QAPP in place. However, approval of the QAPP was soon attained after initial tests. The second deviation pertained to the use of a lower STS concentration than recommended by the vendor (Hach, Loveland, CO) to measure FAC from the anolyte solution, but the use of the lower concentration of STS was clarified and documented by the vendor as being more accurate than what the vendor actually recommended. A third deviation was for the

use of a BSC III for anolyte spray testing when the QAPP described the use of a compact glovebox (CGB), but the positive controls were placed in the CGB and sprayed with SFW instead of the test coupons: both were maintained at the specified temperature and RH settings throughout testing. A fourth deviation was for the exclusion of the qualitative assessment of the presence of residual viable organisms on test coupons from testing, but, after consultation with the Contracting Officer's Representative (COR), this assessment resulted from holdover language from a previous QAPP and was not intended for this evaluation. The last deviation was for having no documentation of the colony counts for five of six positive control (i.e., inoculated test materials not exposed to anolyte) blanks during one testing condition: this deviation could not be addressed since the BBRC has a policy of storing test samples for a finite period of time, and by the time that this oversight was noticed, these positive control blanks had already been disposed. None of these deviations had any significant effect on efficacy determinations for the respective anolyte spray tests.

4.5 QA/QC Reporting

Each assessment and audit was documented in accordance with the QAPP and QMP[1]. For these tests, findings were noted (none believed to be significant) in the data quality audit, but no follow-up corrective action was necessary. The findings were mostly minor data transcription errors requiring some recalculation of efficacy results, but none were gross errors in recording. Copies of the assessment reports were distributed to the EPA and Battelle staff. QA/QC procedures were performed in accordance with the QAPP.

4.6 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in reports. All data were recorded by Battelle staff. The Battelle staff member performing the QC/technical review was involved in the experiments and added his/her initials and the date to a hard copy of the record being reviewed. This hard copy was returned to the Battelle staff member who stored the record.

5.0 Commissioning and Optimization of the EcaFlo® System

5.1 Commissioning

An IET field engineer was on site at the testing facility (Battelle) and (1) inspected the uncrated EcaFlo® system to ensure that nothing had been altered or damaged during shipment; (2) inspected the electrical, water, and ventilation to ensure that all requirements were met as listed in Table 2-1 to operate the EcaFlo® system properly; (3) set up the EcaFlo® system; (4) trained testing facility staff over the course of two days on the operation and maintenance of the EcaFlo® system and the methods for measuring FAC, pH, ORP, and conductivity as it has been done at the IET site; and (5) carefully observed testing facility staff as they generated the following anolyte solutions:

- 1,000 ppm FAC, pH 5
- 1,000 ppm FAC, pH 6
- 1,000 ppm FAC, pH 7
- 2,000 ppm FAC, pH 5
- 2,000 ppm FAC, pH 6
- 2,000 ppm FAC, pH 7
- 3,000 ppm FAC, pH 5
- 3,000 ppm FAC, pH 6
- 3,000 ppm FAC, pH 7

Despite the fact that the EcaFlo® system was set up in a large area (approximately 84 square meters) with sufficient ventilation to remove steam, the off-gassing of the higher HOCl levels (≥ 3,000 ppm) from the waste container warranted additional measures by the testing facility to ventilate the area adequately. The testing area was chosen because of (1) the large footprint of the EcaFlo® system, as there was limited space for this type of system in the containment laboratory; (2) an uninterruptable power supply service was readily available in this area since it had previously been decommissioned; (3) a readily available water source with consistent pressure; (4) powerful ventilation located above the EcaFlo® system; and (5) location on the naïve-side of the testing facility. The availability of these features meant that the EcaFlo® system would not have to be decontaminated and was easily accessible for the field engineer.

The inputs for the EcaFlo® system were easy to obtain. The softener salt for the brine tank was purchased at a local home improvement retailer (previously described in Chapter 2) and was available in many other locations. IET also required the use of muriatic acid (diluted to a 5% hydrochloric acid (HCl)) to descale the electrolytic cell interiors of the EcaFlo® system.

5.2 Optimization and useful life tests

Ten tests were conducted to determine how (1) decreasing the EcaFlo® system flow rate, (2) increasing the power input, (3) increasing the salt concentration, and (4) adjustment (if needed) of other parameters affected levels of FAC, pH, and ORP in the anolyte solutions generated. This part of the evaluation had to achieve targeted FAC levels of 1,000, 2,000, and 3,000 ppm, each at pH levels of 5, 6, and 7, constituting nine tests. The final optimization test was to achieve 3,500 ppm at pH 5.

Due to the reactivity of the anolyte, the concentration of FAC in the solution was expected to degrade over time. Therefore, the levels of FAC, ORP, and pH for each anolyte were measured immediately following generation. After one hour, and after two hours had passed, these values were measured again. The results of this evaluation are shown in Tables 5-1 to 5-4. The targeted FAC and pH levels were achieved at the time of anolyte generation.

The useful-life evaluations for each anolyte showed that gradual degradation occurred over the two- hour span (Figures 5-1 to 5-4) for all anolyte solutions with the exception of one. The anolyte generated at 3,000 ppm, pH 7, retained only 72% of the FAC measured from the time of generation at Time 0 to +2 hours, a significant drop compared to the other anolyte solutions that retained > 91% of the FAC measured over that same span. However, during anolyte decontamination spray testing for this same anolyte solution (3,000 ppm, pH 7), greater than 90% of the FAC was retained during a similar useful-life evaluation (see Chapter 8, Table 8-4). Although no experimental anomalies were noted during the test in which FAC dropped to 72% of its original value, this data point is most likely an outlier due to an experimental error.

Table 5-1. Optimization and useful-life of 1,000 ppm FAC anolyte

Target FAC[1], pH	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (avg. mS)	Flow Rate (Lph)	Actual FAC, ORP[2], pH at Time 0	Actual FAC, ORP, pH at +1 hr	Actual FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
1000, 5	86.1	23.1	19.5	12.5	83.4	1097.5, 1058, 5.06	1080, 1078, 4.87	1042.5,1102, 4.63	37.9
1000, 6	86.1	23.1	19.7	12.5	83.4	1047.5, 987, 6.02	1042, 990, 5.99	1025, 1002, 5.97	37.9
1000, 7	86.1	23.3	19.5	12.6	85.3	940, 919, 6.95	940, 925, 6.92	907.5, 929, 6.90	45.5

[1] Reported as ppm.
[2] Reported as mV.

Table 5-2. Optimization and useful-life of 2,000 ppm FAC anolyte

Target FAC[1], pH	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (avg. mS)	Flow Rate (Lph)	Actual FAC, ORP[2], pH at Time 0	Actual FAC, ORP, pH at +1 hr	Actual FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
2000, 5	79.2	14.1	28	22.4	68.2	2055, 1076, 5.03	2057, 1114, 4.47	2049, 1118, 4.35	22.7
2000, 6	92.2	13.6	35	22.1	72.0	2080, 1010, 6.03	1950, 1029 5.77	1962, 1028, 5.76	26.9
2000, 7	92.2	13.6	35	22.4	72.0	2087, 918, 7.03	1905, 929, 7.01	1907, 933, 7.00	22.7

[1] Reported as ppm.
[2] Reported as mV.

Table 5-3. Optimization and useful-life of 3,000 ppm FAC anolyte

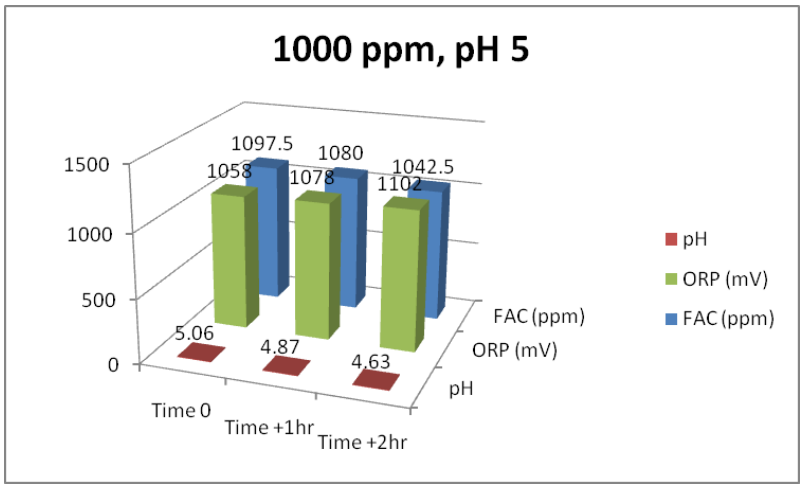
Target FAC[1], pH	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (avg. mS)	Flow Rate (Lph)	Actual FAC, ORP[2], pH at Time 0	Actual FAC, ORP, pH at +1 hr	Actual FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
3000, 5	105	9.7	95	41.8	68.2	2925, 1089, 5.01	2970, 1110, 4.73	2675, 1113, 4.63	28.4
3000, 6	105	9.3	95	44.2	68.2	3252, 1018, 6.01	3220, 1028 5.82	3277, 1036, 5.73	28.4
3000, 7	104.8	9.7	98	45.1	68.2	2800, 937, 6.96	2500, 941, 6.90	2012, 945, 6.79	32.6

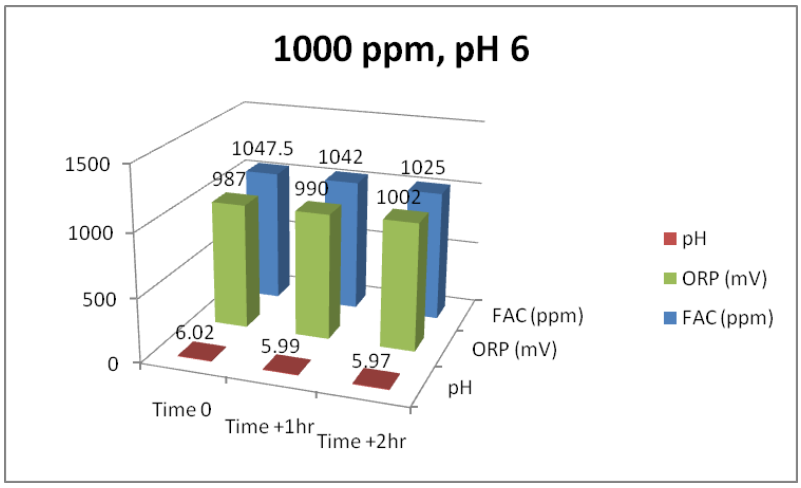
[1] Reported as ppm.
[2] Reported as mV.

Table 5-4. Optimization and useful-life of 3,500 ppm FAC anolyte

Target FAC[1], pH	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (avg. mS)	Flow Rate (Lph)	Actual FAC, ORP[2], pH at Time 0	Actual FAC, ORP, pH at +1 hr	Actual FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
3500, 5	105.6	9.6	95	46.7	72.0	3515, 1086, 5.01	3480, 1106, 4.71	3280, 1080, 4.70	28.4

[1] Reported as ppm.
[2] Reported as mV.





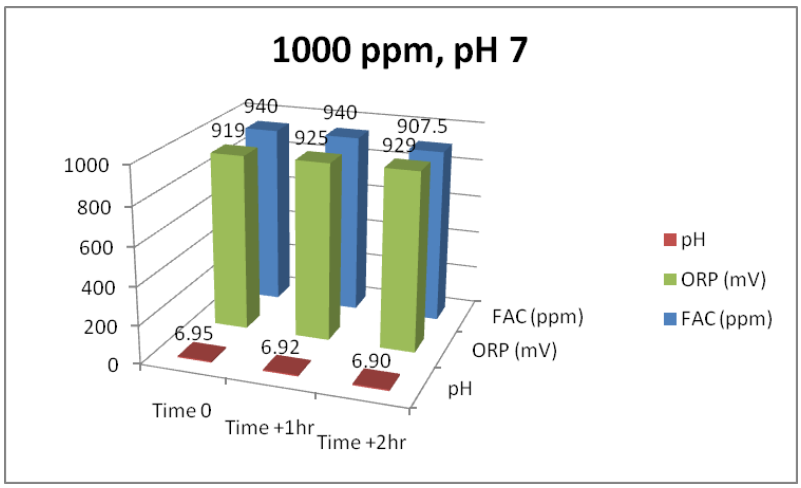


Figure 5-1. Useful-life measurements for 1,000 ppm FAC anolyte at pH 5, 6, and 7

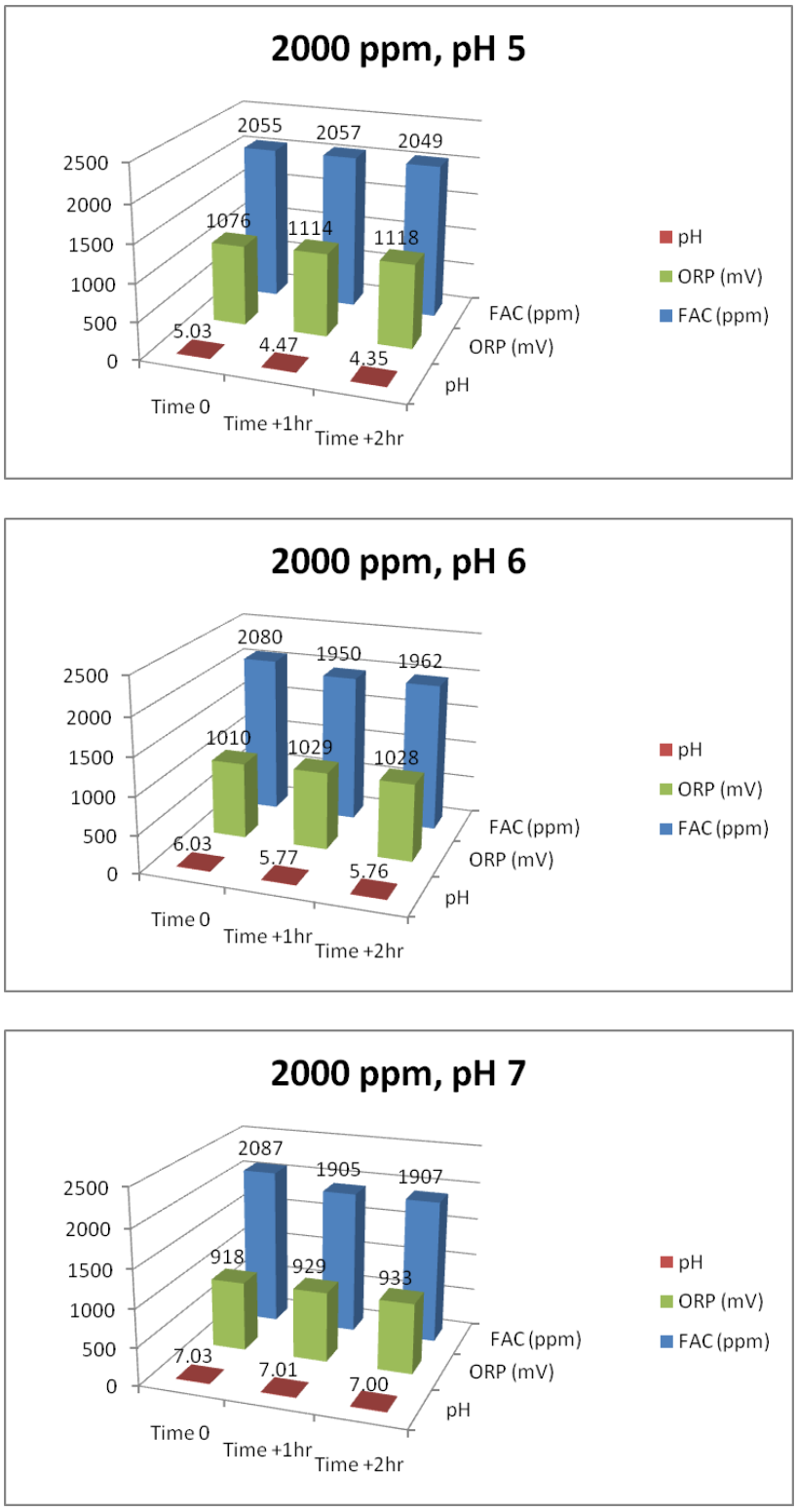


Figure 5-2. Useful-life measurements for 2,000 ppm FAC anolyte, pH 5, 6, and 7

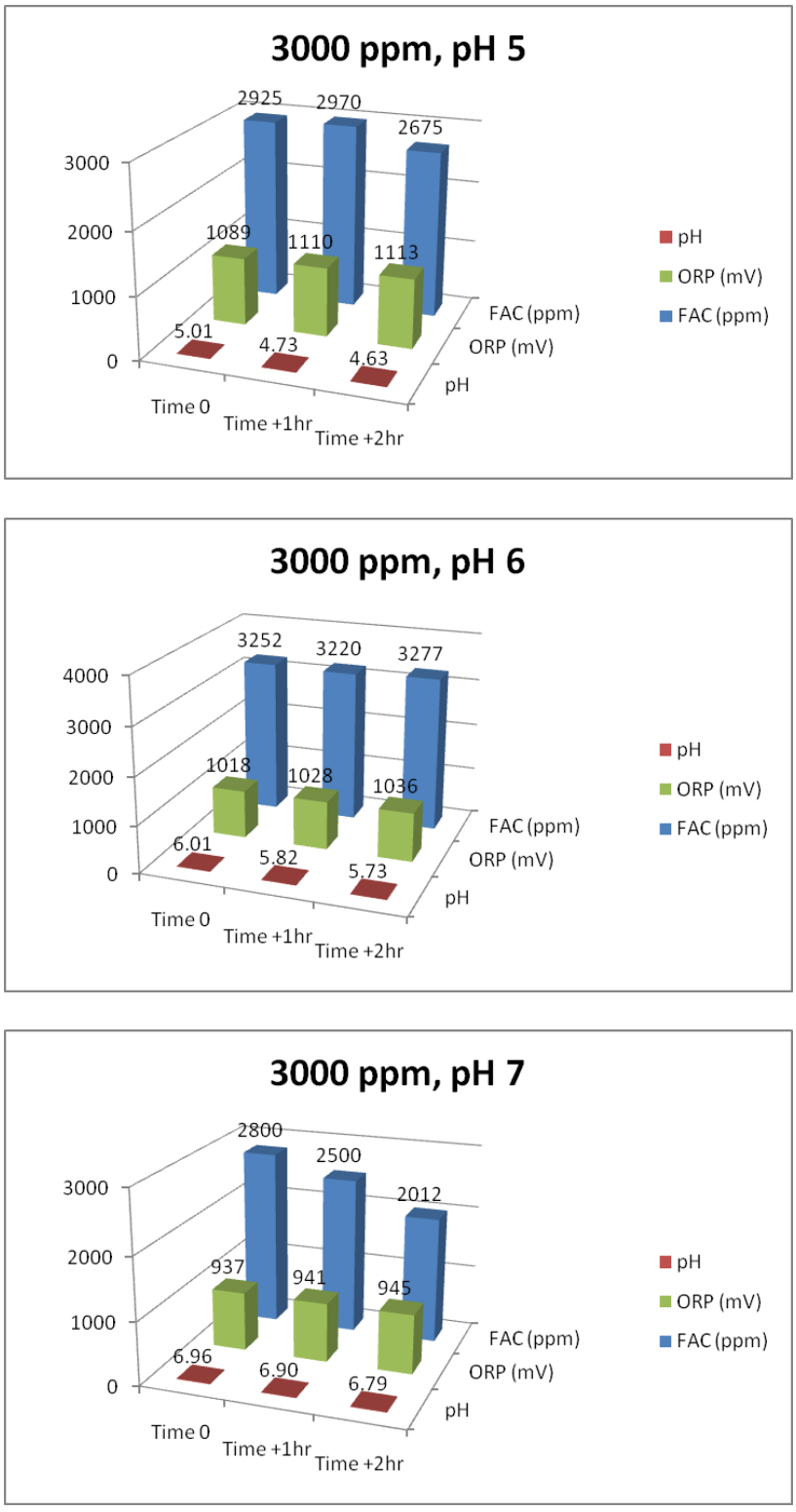


Figure 5-3. Useful-life measurements for 3,000 ppm FAC anolyte, pH 5, 6, and 7

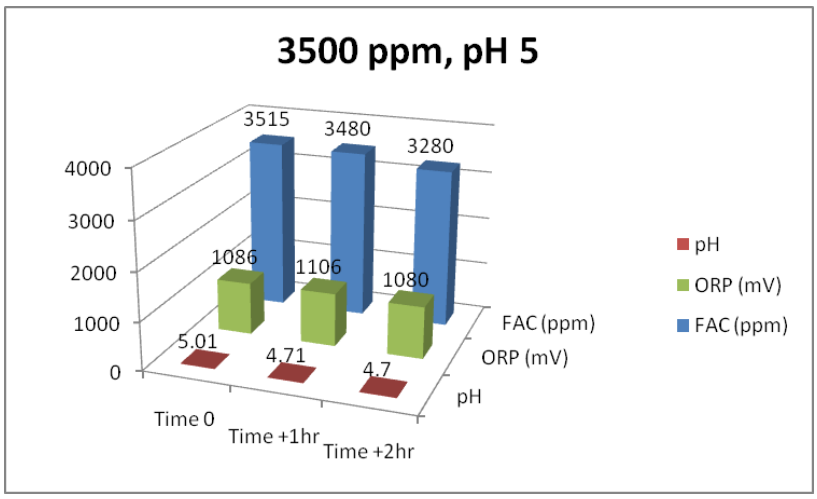


Figure 5-4. Useful-life measurements for 3,500 ppm FAC anolyte, pH 5, 6, and 7

6.0 Anolyte Solution Test Results for 3,000 ppm FAC, pH 5

6.1 QC Results

The anolyte solution with a target of 3,000 ppm FAC, pH 5, was sprayed at Time 0 and at +30 minutes for a total of two spray applications with a total contact time of 60 minutes (i.e., anolyte allowed to dwell for an additional 30 minutes after the +30 minute spray). In testing of this anolyte batch, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values for *B. anthracis* spores ranged from 3.03 to 64.98%, with the lowest recovery occurring on painted wallboard paper, and the highest recovery occurring on galvanized metal. Positive control recovery values for *B. subtilis* spores ranged from 2.94 to 46.80%, with the lowest recovery occurring on bare pine wood, and the highest recovery occurring on industrial carpet. Refer to Tables 6-1 and 6-2.

In testing of the 3,000 ppm FAC, pH 5 anolyte (60 min contact time, two total spray applications), all procedural and laboratory blanks met the criterion of no observed CFU.

Spike control samples were taken from the spore suspension on each day of testing and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for *B. anthracis* and *B. subtilis* testing for this anolyte batch were 1.19×10^8/coupon and 1.17×10^8/coupon, respectively.

6.2 Decontamination Efficacy

The decontamination efficacy of 3,000 ppm FAC, pH 5 anolyte was evaluated for *B. anthracis* and *B. subtilis* on six building material surfaces. The decontamination efficacy of 3,000 ppm FAC, pH 5 anolyte (60 min contact time, two total spray applications) for *B. anthracis* was less than 6 log reduction on all six materials, as shown in Table 6-1 and summarized in Table 6-3. The highest efficacies occurred on decorative laminate (5.95), galvanized metal (4.58), and glass (4.55), all non-porous materials. Lower efficacies occurred with industrial carpet (0.58), painted wallboard paper (2.57), and bare pine wood (2.13), all porous materials.

Fairly similar results were seen for *B. subtilis*, as shown in Table 6-2 (summarized in Table 6-3), with the highest efficacies on decorative laminate (5.95), galvanized metal (greater than or equal to 7.71), and glass (6.14), all non-porous materials. The efficacy results for galvanized metal were equivalent to complete inactivation within the detection limit. Lower efficacies occurred with industrial carpet (0.73), painted-wallboard paper (1.71), and bare pine wood (0.30).

Table 6-1. Inactivation of *Bacillus anthracis* spores—3,000 ppm FAC, pH 5 anolyte, by material (60 minute contact with re-application at 30 minutes for two total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.19×10^8	7.84 ± 0.060	58.80 ± 7.81	-
Test Coupons[c]	1.19×10^8	7.26 ± 0.27	17.85 ± 10.86	0.58 ± 0.24
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.19×10^8	7.86 ± 0.04	60.46 ± 6.03	-
Test Coupons	1.19×10^8	1.91 ± 1.19	< 0.01	5.95 ± 1.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.19×10^8	7.88 ± 0.08	64.98 ± 11.18	-
Test Coupons	1.19×10^8	3.30 ± 0.12	< 0.01	4.58 ± 0.12
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.19×10^8	6.55 ± 0.09	3.03 ± 0.58	-
Test Coupons	1.19×10^8	3.98 ± 0.19	< 0.01	2.57 ± 0.18
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.19×10^8	6.83 ± 0.07	5.72 ± 0.96	-
Test Coupons	1.19×10^8	4.70 ± 0.29	< 0.01	2.13 ± 0.26
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.19×10^8	7.85 ± 0.04	59.72 ± 5.60	-
Test Coupons	1.19×10^8	3.30 ± 0.15	< 0.01	4.55 ± 0.14
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 6-2. Inactivation of *Bacillus subtilis* spores—3,000 ppm FAC, pH 5 anolyte, by material (60 minute contact with re-application at 30 minutes for two total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.17×10^8	7.73 ± 0.07	46.80 ± 7.10	-
Test Coupons[c]	1.17×10^8	7.00 ± 0.05	8.67 ± 0.98	0.73 ± 0.07
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.17×10^8	7.70 ± 0.05	43.45 ± 5.05	-
Test Coupons	1.17×10^8	1.75 ± 1.00	< 0.01	5.95 ± 0.88
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.17×10^8	7.71 ± 0.08	44.37 ± 8.06	-
Test Coupons	1.17×10^8	0	0	≥ 7.71 ± 0.07
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.17×10^8	6.81 ± 0.17	5.92 ± 2.28	-
Test Coupons	1.17×10^8	5.10 ± 0.73	0.28± 0.34	1.71 ± 0.65
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.17×10^8	6.52 ± 0.12	2.94 ± 0.96	-
Test Coupons	1.17×10^8	6.22 ± 0.11	1.44 ± 0.30	0.30 ± 0.14
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.17×10^8	7.38 ± 0.35	25.38 ± 16.05	-
Test Coupons	1.17×10^8	1.24 ± 1.14	< 0.01	6.14 ± 1.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 6-3. Summary of mean efficacy (log reduction) values for 3,000 ppm FAC, pH 5 anolyte (60 minute contact with re-application of spray at +30 minutes for two total applications)

Test Material	Efficacy for *B. anthracis* (Ames)	Efficacy for *B. subtilis*
Industrial Carpet	0.58	0.73
Decorative Laminate	5.95	5.95
Galvanized Metal	4.58	7.71[a]
Painted Wallboard Paper	2.57	1.71
Bare Pine Wood	2.13	0.30
Glass	4.55	6.14

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/material.

6.3 Damage to Coupons

No visible damage was observed on the test materials after the 60 min contact time and two total spray applications with this anolyte (3,000 ppm FAC, pH 5).

6.4 Other Factors

6.4.1 Anolyte Useful-Life

The measurements listed in Table 6-4 and graphed in Figure 6-1 show an FAC useful-life of greater than 95% and a pH useful-life of greater than 92% from the readings made at the time of anolyte generation (Time 0).

Table 6-4. Measurements and useful-life of 3000 ppm FAC, pH 5 anolyte solution

Flow Rate (Lph)	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (mS)	Target FAC[1], pH	FAC, ORP[2], pH at Time 0	FAC, ORP, pH at +1 hr	FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
75.8	105	9.70	92	41.7	3000, 5	2803, 1086, 5.01	2787, 1113, 4.63	2683, 1110, 4.62	26.2

[1] Reported as ppm.
[2] Reported as mV.

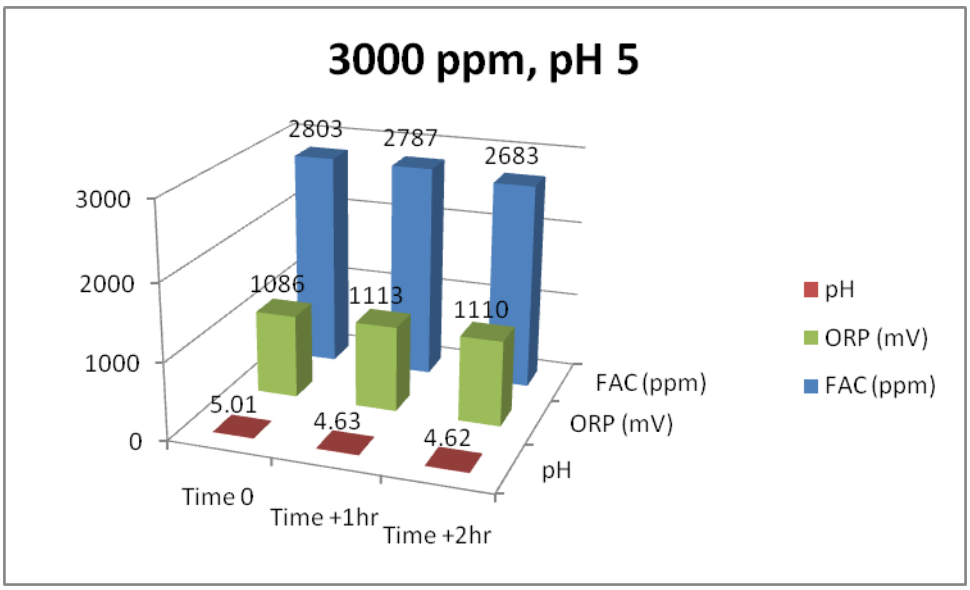


Figure 6-1. Measurements and useful-life for 3,000 ppm FAC, pH 5 anolyte

6.4.2 Anolyte Spray Deposition

The anolyte was applied from a distance of 30.5 cm (12 inches) to the horizontally-oriented materials until the materials were fully wetted. Re-application of the anolyte was made on all coupon surfaces at 30 minutes after the initial application, for a total of two applications. At 60 minutes after the initial application, each material coupon was placed in the 50 mL conical tube that also served to collect excess anolyte runoff.

Prior to decontamination testing, to assess the amount of anolyte deposited via spraying, triplicate coupons of each test material were weighed prior to application of the anolyte in the trial runs, and these values were recorded. The triplicate coupons were then sprayed with anolyte until fully wetted in their horizontal orientations, re-application was made at 30 minutes contact time for a total of two applications, and after 60 minutes contact time each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the anolyte from each of the test materials is shown in Table 6-5. The total averaged value (0.22 g) over all six materials was then used to estimate the amount of STS needed to effectively neutralize the anolyte under this testing condition.

Table 6-5. Deposition/runoff weight of 3,000 ppm FAC, pH 5 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Test Material	Average Deposition/Runoff Weight (g)
Industrial Carpet	0.24
Decorative Laminate	0.22
Galvanized Metal	0.20
Painted Wallboard Paper	0.13
Bare Pine Wood	0.25
Glass	0.25
Average	*0.22*

6.4.3 Neutralization Methodology

Neutralization of the 3,000 ppm FAC, pH 5 anolyte was achieved with STS. The concentrations of STS tried during the neutralization trials were 0.5, 1.0, and 1.5% in the extraction solution. These STS concentrations were based on historical data.

The results of the neutralization trials are shown in Tables 6-6 and 6-7. From these trials, 0.5% STS was determined to be sufficient for neutralization of the 3,000 ppm FAC, pH 5 anolyte for *B. anthracis* and 1.0% STS for *B. subtilis*.

Table 6-6. Neutralization testing with *Bacillus anthracis* spores with 3,000 ppm FAC, pH 5 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	1.08×10^8	1.83×10^3	0.0020
Anolyte + PBS + Triton X-100 + Spores[ab]	1.08×10^8	4.22×10^4	0.045
PBS + Triton X-100 + Spores (Control)[b]	1.08×10^8	9.29×10^7	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	1.08×10^8	9.82×10^7	105.72
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	1.08×10^8	8.32×10^7	89.59
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	1.08×10^8	1.03×10^8	111.22

[a] Anolyte volume of 0.22 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.22 mL (10 mL PBS/Triton X-100/STS + 0.22 mL anolyte).

Table 6-7. Neutralization testing with *Bacillus subtilis* spores with 3,000 ppm FAC, pH 5 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	7.93×10^7	0	0
Anolyte + PBS + Triton X-100 + Spores[ab]	7.93×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	7.93×10^7	1.01×10^8	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	7.93×10^7	7.54×10^7	74.57
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	7.93×10^7	9.29×10^7	91.89
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	7.93×10^7	8.89×10^7	87.95

[a] Anolyte volume of 0.22 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.22 mL (10 mL PBS/Triton X-100/STS + 0.22 mL anolyte).

7.0 Anolyte Solution Test Results for 3,000 ppm FAC, pH 6

7.1 QC Results

The anolyte solution with a target of 3,000 ppm FAC, pH 6, was sprayed at Time 0 and at +30 minutes for a total of two spray applications with a total contact time of 60 minutes (i.e., anolyte allowed to dwell for an additional 30 minutes after the +30 minute spray). In testing of this anolyte, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values for *B. anthracis* spores ranged from 6.49 to 112.51%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on industrial carpet. Positive control recovery values for *B. subtilis* spores ranged from 3.46 to 53.79%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on galvanized metal. Refer to Tables 7-1 and 7-2

In testing of the 3,000 ppm FAC, pH 6 anolyte (60 min contact time, two total spray applications), all procedural and laboratory blanks met the criterion of no observed CFU.

Spike control samples were taken from the spore suspension on each day of testing and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for *B. anthracis* and *B. subtilis* testing for this anolyte batch were 1.00×10^8/coupon and 1.14×10^8/coupon, respectively.

7.2 Decontamination Efficacy

The decontamination efficacy of 3000 ppm FAC, pH 6 anolyte was evaluated for *B. anthracis* and *B. subtilis* on six building material surfaces. The decontamination efficacy of 3,000 ppm FAC, pH 6 anolyte (60 min contact time, two total spray applications) for *B. anthracis* was greater than or equal to 7 log reduction on three of the six materials which was equivalent to complete inactivation within the detectable limit as shown in Table 7-1 and summarized in Table 7-3. The highest efficacies occurred on decorative laminate (≥ 7.55) and glass (≥ 7.83), with near-complete inactivation observed on galvanized metal (7.46), all non-porous materials. Lower efficacies occurred with industrial carpet (0.26), painted wallboard paper (2.18), and bare pine wood (0.68), all porous materials.

Similar results were seen for *B. subtilis*, as shown in Table 7-2 and summarized in Table 7-3, with ≥ 7 log reduction on decorative laminate (≥ 7.57), galvanized metal (≥ 7.79), and glass (≥ 7.75); all are non-porous materials, with efficacy equivalent to complete inactivation within the detection limit. Lower efficacies occurred with industrial carpet (0.21), painted wallboard paper (2.51), and bare pine wood (0.47).

Table 7-1. Inactivation of *Bacillus anthracis* spores—3,000 ppm FAC, pH 6 anolyte by material (60 minute contact with re-application at 30 minutes for two total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.00×10^8	8.05 ± 0.03	112.51 ± 7.34	-
Test Coupons[c]	1.00×10^8	7.79 ± 0.09	63.38 ± 14.38	0.26 ± 0.08
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.00×10^8	7.55 ± 0.26	40.55 ± 19.39	-
Test Coupons	1.00×10^8	0	0	≥7.55 ± 0.23
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.00×10^8	7.83 ± 0.05	67.39 ± 8.33	-
Test Coupons	1.00×10^8	0.37 ± 0.82	< 0.01	7.46 ± 0.72
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.00×10^8	7.62 ± 0.07	42.45 ± 7.53	-
Test Coupons	1.00×10^8	5.44 ± 0.34	0.34 ± 0.22	2.18 ± 0.30
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.00×10^8	6.80 ± 0.10	6.49 ± 1.53	-
Test Coupons	1.00×10^8	6.12 ± 0.20	1.42 ± 0.61	0.68 ± 0.19
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.00×10^8	7.83 ± 0.08	68.28 ± 11.68	-
Test Coupons	1.00×10^8	0	0	≥7.83 ± 0.07
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 7-2. Inactivation of *Bacillus subtilis* spores—3,000 ppm FAC, pH 6 anolyte by material (60 minute contact with re-application at 30 minutes for two total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.14×10^8	7.73 ± 0.04	47.50 ± 5.12	-
Test Coupons[c]	1.14×10^8	7.52 ± 0.05	29.30 ± 3.68	0.21 ± 0.06
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.14×10^8	7.57 ± 0.07	32.90 ± 5.15	-
Test Coupons	1.14×10^8	0	0	≥7.57 ± 0.06
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.14×10^8	7.79 ± 0.04	53.79 ± 5.43	-
Test Coupons	1.14×10^8	0	0	≥7.79 ± 0.04
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.14×10^8	6.77 ± 0.07	5.22 ± 0.90	-
Test Coupons	1.14×10^8	4.26 ± 0.28	0.019 ± 0.012	2.51 ± 0.25
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.14×10^8	6.59 ± 0.06	3.46 ± 0.52	-
Test Coupons	1.14×10^8	6.12 ± 0.08	1.17 ± 0.22	0.47 ± 0.09
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.14×10^8	7.75 ± 0.05	49.87 ± 5.93	-
Test Coupons	1.14×10^8	0	0	≥7.75 ± 0.04
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 7-3. Summary of mean efficacy (log reduction) values for 3,000 ppm FAC, pH 6 anolyte (60 minute contact with re-application of spray at +30 minutes for two total applications)

Test Material	Efficacy for *B. anthracis* (Ames)	Efficacy for *B. subtilis*
Industrial Carpet	0.26	0.21
Decorative Laminate	7.55[a]	7.57[a]
Galvanized Metal	7.46	7.79[a]
Painted Wallboard Paper	2.18	2.51
Bare Pine Wood	0.68	0.47
Glass	7.83[a]	7.75[a]

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/coupon.

7.3 Damage to Coupons

No visible damage was observed on the test materials after the 60 min contact time and two total spray applications with this anolyte (3,000 ppm FAC, pH 6).

7.4 Other Factors

7.4.1 Anolyte Useful-Life

The measurements listed in Table 7-4 and graphed in Figure 7-1 show an FAC useful-life of greater than 98% and a pH useful-life of greater than 96% from the readings made at the time of anolyte generation (Time 0).

Table 7-4. Measurements and useful-life of 3000 ppm FAC, pH 6 anolyte solution

Flow Rate (Lph)	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (mS)	Target FAC[1], pH	FAC, ORP[2], pH at Time 0	FAC, ORP, pH at +1 hr	FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
75.8	105	9.70	92	44.5	3000, 6	2850, 1008, 5.97	2827, 1017, 5.75	2818, 1028, 5.73	26.8

[1] Reported as ppm.
[2] Reported as mV.

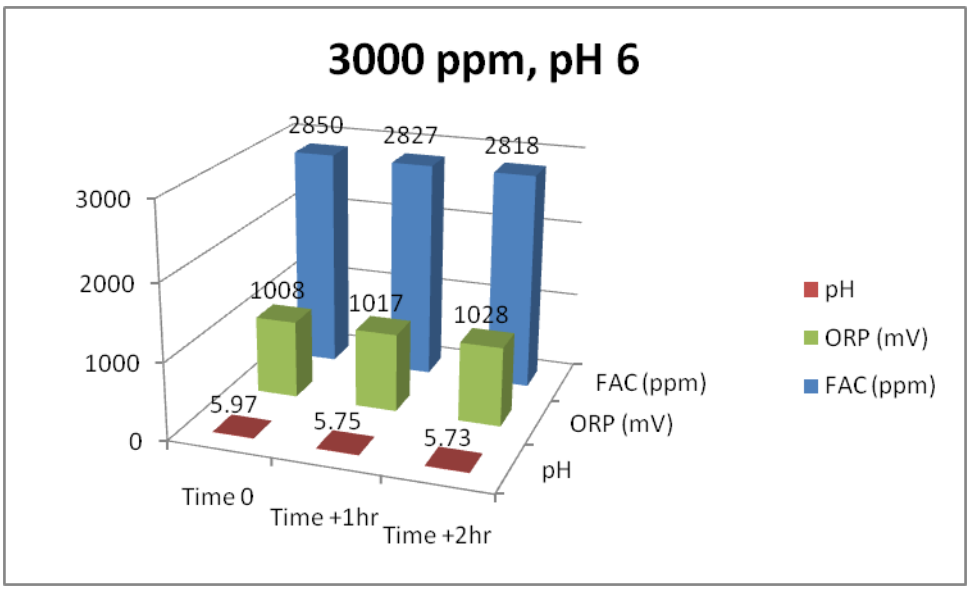


Figure 7-1. Measurements and useful-life for 3,000 ppm FAC, pH 6 anolyte

7.4.2 Anolyte Spray Deposition

The anolyte was applied from a distance of 30.5 cm (12 inches) to the horizontally-oriented materials until the materials were fully wetted. Re-application of the anolyte was made on all coupon surfaces at 30 minutes after the initial application, for a total of two applications. At 60 minutes after the initial application, each material coupon was placed in the 50 mL conical tube that also served to collect excess anolyte runoff.

Prior to decontamination testing, to assess the amount of anolyte deposited via spraying, triplicate coupons of each test material were weighed prior to application of the anolyte in the trial runs, and these values were recorded. These triplicate coupons were then sprayed with anolyte until fully wetted in their horizontal orientations, re-application was made at 30 minutes contact time for a total of two applications, and after 60 minutes contact time each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the anolyte from each of the test materials is shown in Table 7-5. The total averaged value (0.21 g) over all six materials was then used to estimate the amount of STS needed to effectively neutralize the anolyte under this testing condition.

Table 7-5. Deposition/runoff weight of 3,000 ppm FAC, pH 6 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Test Material	Average Deposition/Runoff Weight (g)
Industrial Carpet	0.28
Decorative Laminate	0.16
Galvanized Metal	0.21
Painted Wallboard Paper	0.17
Bare Pine Wood	0.28
Glass	0.15
Average	*0.21*

7.4.3 Neutralization Methodology

Neutralization of the 3,000 ppm FAC, pH 6 anolyte was achieved with STS. The concentrations of STS tried during the neutralization panels were 0.5, 1.0, and 1.5% in the extraction solution. These STS concentrations were based on historical data.

The results of the neutralization panels are shown in Tables 6-6 and 6-7. From these panels, 0.5% STS was determined to be sufficient for neutralization of the 3,000 ppm FAC, pH 6 anolyte for *B. anthracis* and 1.0% STS for *B. subtilis*.

Table 7-6. Neutralization testing with *Bacillus anthracis* spores with 3,000 ppm FAC, pH 6 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	1.08×10^8	1.70×10^3	0.0016
Anolyte + PBS + Triton X-100 + Spores[ab]	1.08×10^8	8.28×10^4	0.078
PBS + Triton X-100 + Spores (Control)[b]	1.08×10^8	1.06×10^8	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	1.08×10^8	1.02×10^8	96.64
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	1.08×10^8	8.72×10^7	82.29
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	1.08×10^8	8.73×10^7	82.32

[a] Anolyte volume of 0.21 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.21 mL (10 mL PBS/Triton X-100/STS + 0.21 mL anolyte).

Table 7-7. Neutralization testing with *Bacillus subtilis* spores with 3,000 ppm FAC, pH 6 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	7.93×10^7	0	0
Anolyte + PBS + Triton X-100 + Spores[ab]	7.93×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	7.93×10^7	9.41×10^7	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	7.93×10^7	9.44×10^7	100.29
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	7.93×10^7	1.06×10^8	112.12
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	7.93×10^7	1.03×10^8	109.22

[a] Anolyte volume of 0.21 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.21 mL (10 mL PBS/Triton X-100/STS + 0.21 mL anolyte).

8.0 Anolyte Solution Test Results for 3,000 ppm FAC, pH 7

8.1 QC Results

The anolyte solution with a target of 3,000 ppm FAC, pH 7, was sprayed at Time 0 and at +30 minutes for a total of two spray applications with a total contact time of 60 minutes (i.e., anolyte allowed to dwell for an additional 30 minutes after the +30 minute spray). In testing of this anolyte, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values for *B. anthracis* spores ranged from 16.29 to 104.95%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on industrial carpet. Positive control recovery values for *B. subtilis* spores ranged from 4.63 to 68.97%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on galvanized metal. Refer to Tables 8-1 and 8-2.

In testing of the 3,000 ppm FAC, pH 7 anolyte (60 min contact time, two total spray applications), all procedural and laboratory blanks met the criterion of no observed CFU.

Spike control samples were taken from the spore suspension on each day of testing and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for *B. anthracis* and *B. subtilis* testing for this anolyte batch were 1.04×10^8/coupon and 1.40×10^8/coupon, respectively.

8.2 Decontamination Efficacy

The decontamination efficacy of 3,000 ppm FAC, pH 7 anolyte was evaluated for *B. anthracis* and *B. subtilis* on six building material surfaces. The decontamination efficacy of 3,000 ppm FAC, pH 7 anolyte (60 min contact time, two total applications) for *B. anthracis* was ≥ 7 log reduction on decorative laminate (≥ 7.28) and glass (≥ 7.93), equivalent to complete inactivation within the detection limit as shown in Table 8-1 and summarized in Table 8-3. Near-complete inactivation was observed on galvanized metal (7.61), so the highest efficacies were seen on all the non-porous materials. Lower efficacies occurred with industrial carpet (0.31), painted wallboard paper (2.62), and bare pine wood (1.02), all porous materials.

Similar results were seen for *B. subtilis*, as shown in Table 8-2 and summarized in Table 8-3, with ≥ 7 log reduction on two of six materials, equivalent to complete inactivation within the detection limit on decorative laminate (≥ 6.91), galvanized metal (≥ 7.98), and glass (≥ 7.85), all non-porous materials. Lower efficacies occurred with industrial carpet (0.61), painted wallboard paper (3.01), and bare pine wood (0.67).

Table 8-1. Inactivation of *Bacillus anthracis* spores—3,000 ppm FAC, pH 7 anolyte, by material (60 minute contact with re-application at 30 minutes for two total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.04×10^8	8.04 ± 0.05	104.95 ± 12.57	-
Test Coupons[c]	1.04×10^8	7.73 ± 0.04	51.96 ± 5.12	0.31 ± 0.06
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.04×10^8	7.89 ± 0.04	75.47 ± 7.53	-
Test Coupons	1.04×10^8	0.61 ± 0.84	< 0.01	7.28 ± 0.74
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.04×10^8	7.92 ± 0.08	80.32 ± 14.10	-
Test Coupons	1.04×10^8	0.31 ± 0.69	< 0.01	7.61 ± 0.60
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.04×10^8	7.85 ± 0.06	68.53 ± 9.81	-
Test Coupons	1.04×10^8	5.23 ± 0.73	0.42 ± 0.65	2.62 ± 0.64
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.04×10^8	7.16 ± 0.25	16.29 ± 12.26	-
Test Coupons	1.04×10^8	6.14 ± 0.07	1.35 ± 0.21	1.02 ± 0.23
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.04×10^8	7.93 ± 0.05	82.09 ± 8.34	-
Test Coupons	1.04×10^8	0	0	≥7.93 ± 0.04
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 8-2. Inactivation of *Bacillus subtilis* spores—3,000 ppm FAC, pH 7 anolyte, by material (60 minute contact with re-application at 30 minutes for two total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.40×10^8	7.77 ± 0.08	42.30 ± 7.49	-
Test Coupons[c]	1.40×10^8	7.16 ± 0.13	10.74 ± 3.00	0.61 ± 0.13
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.40×10^8	7.87 ± 0.02	53.10± 2.07	-
Test Coupons	1.40×10^8	0.96 ± 1.32	0	6.91 ± 1.16
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.40×10^8	7.98 ± 0.05	68.97 ± 7.82	-
Test Coupons	1.40×10^8	0	0	≥7.98 ± 0.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.40×10^8	7.51 ± 0.33	28.57 ± 20.18	-
Test Coupons	1.40×10^8	4.49 ± 0.86	0.10 ± 0.19	3.01 ± 0.80
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.40×10^8	6.76 ± 0.22	4.63 ± 2.87	-
Test Coupons	1.40×10^8	6.09 ± 0.17	0.95 ± 0.45	0.67 ± 0.25
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.40×10^8	7.85 ± 0.13	52.31 ± 13.83	-
Test Coupons	1.40×10^8	0	0	≥7.85 ± 0.11
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 8-3. Summary of mean efficacy (log reduction) values for 3,000 ppm FAC, pH 7 anolyte (60 minute contact with re-application of spray at +30 minutes for two total applications)

Test Material	Efficacy for *B. anthracis* (Ames)	Efficacy for *B. subtilis*
Industrial Carpet	0.31	0.61
Decorative Laminate	7.28[a]	6.91[a]
Galvanized Metal	7.61	7.98[a]
Painted Wallboard Paper	2.62	3.01
Bare Pine Wood	1.02	0.67
Glass	7.93[a]	7.85[a]

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/material.

8.3 Damage to Coupons

No visible damage was observed on the test materials after the 60 min contact time and two total spray applications with this anolyte (3,000 ppm FAC, pH 7).

8.4 Other Factors

8.4.1 Anolyte Useful-Life

The measurements listed in Table 8-4 and graphed in Figure 8-1 show an FAC useful-life of greater than 90% and a pH useful-life of greater than 98% from the readings made at the time of anolyte generation (Time 0).

Table 8-4. Measurements and useful-life of 3000 ppm FAC, pH 7 anolyte solution

Flow Rate (Lph)	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (mS)	Target FAC[1], pH	FAC, ORP[2], pH at Time 0	FAC, ORP, pH at +1 hr	FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
68.2	75	8.50	95	45.5	3000, 7	2820, 933, 6.94	2595, 948, 6.88	2563, 955, 6.87	28.4

[1] Reported as ppm.
[2] Reported as mV.

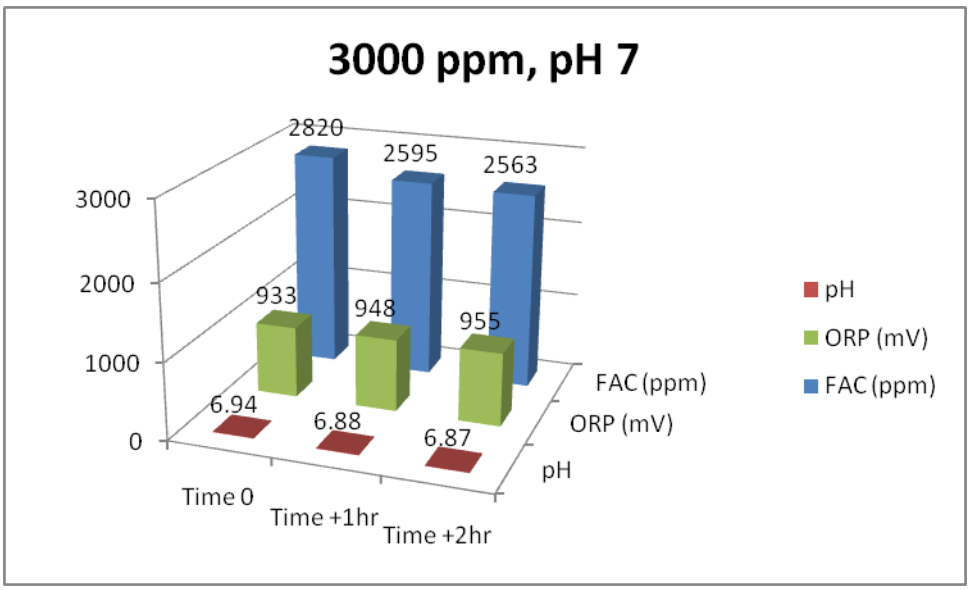


Figure 8-1. Measurements and useful-life for 3,000 ppm FAC, pH 7 anolyte

8.4.2 Anolyte Spray Deposition

The anolyte was applied from a distance of 30.5 cm (12 inches) to the horizontally-oriented materials until the materials were fully wetted. Re-application of the anolyte was made on all coupon surfaces at 30 minutes after the initial application, for a total of two applications. At 60 minutes after the initial application, each material coupon was placed in the 50 mL conical tube that also served to collect excess anolyte runoff.

Prior to decontamination testing, to assess the amount of anolyte deposited via spraying, triplicate coupons of each test material were weighed prior to application of the anolyte in the trial runs, and these values were recorded. The triplicate coupons were then sprayed with anolyte until fully wetted in their horizontal orientations, re-application was made at 30 minutes contact time for a total of two applications, and after 60 minutes contact time each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the anolyte from each of the test materials is shown in Table 8-5. The total averaged value (0.22 g) over all six materials was then used to estimate the amount of STS needed to neutralize the anolyte effectively under this testing condition.

Table 8-5. Deposition/runoff weight of 3,000 ppm FAC, pH 7 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Test Material	Average Deposition/Runoff Weight (g)
Industrial Carpet	0.22
Decorative Laminate	0.16
Galvanized Metal	0.18
Painted Wallboard Paper	0.18
Bare Pine Wood	0.28
Glass	0.29
Average	*0.22*

8.4.3 Neutralization Methodology

Neutralization of the 3,000 ppm FAC, pH 7 anolyte was achieved with STS. The concentrations of STS tried during the neutralization panels were 0.5, 1.0, and 1.5% in the extraction solution. These STS concentrations were based on historical data.

The results of the neutralization panels are shown in Tables 8-6 and 8-7. From these panels, 1.0% STS was determined to be sufficient for neutralization of the 3,000 ppm FAC, pH 7 anolyte for both *B. anthracis* and *B. subtilis*.

Table 8-6. Neutralization testing with *Bacillus anthracis* spores with 3,000 ppm FAC, pH 7 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	1.08×10^8	4.50×10^3	0.0045
Anolyte + PBS + Triton X-100 + Spores[ab]	1.08×10^8	3.33×10^3	0.0033
PBS + Triton X-100 + Spores (Control)[b]	1.08×10^8	1.00×10^8	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	1.08×10^8	9.62×10^7	95.98
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	1.08×10^8	1.01×10^8	100.60
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	1.08×10^8	8.46×10^7	84.39

[a] Anolyte volume of 0.22 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.22 mL (10 mL PBS/Triton X-100/STS + 0.22 mL anolyte).

Table 8-7. Neutralization testing with *Bacillus subtilis* spores with 3,000 ppm FAC, pH 7 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	7.93×10^7	0	0
Anolyte + PBS + Triton X-100 + Spores[ab]	7.93×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	7.93×10^7	1.06×10^8	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	7.93×10^7	8.91×10^7	83.67
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	7.93×10^7	9.49×10^7	89.20
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	7.93×10^7	7.99×10^7	75.03

[a] Anolyte volume of 0.22 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.
[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.22 mL (10 mL PBS/Triton X-100/STS + 0.22 mL anolyte).

9.0 Anolyte Solution Test Results for 3,500 ppm FAC, pH 5, 60 Minute Contact

9.1 QC Results

The anolyte solution with a target of 3,500 ppm FAC, pH 5, was sprayed at Time 0 and at +30 minutes for a total of two spray applications with a total contact time of 60 minutes (i.e., anolyte allowed to dwell for an additional 30 minutes after the +30 minute spray). In testing of this anolyte, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values for *B. anthracis* spores ranged from 8.48 to 84.46%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on industrial carpet. Positive control recovery values for *B. subtilis* spores ranged from 4.35 to 67.95%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on industrial carpet. Refer to Tables 9-1 and 9-2.

In testing of the 3,500 ppm FAC, pH 5 anolyte (60 min contact time, two total spray applications), all procedural and laboratory blanks met the criterion of no observed CFU.

Spike control samples were taken from the spore suspension on each day of testing and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for *B. anthracis* and *B. subtilis* testing for this anolyte batch were 1.39×10^8/coupon and 9.83×10^7/coupon, respectively.

9.2 Decontamination Efficacy

The decontamination efficacy of 3,500 ppm FAC, pH 5 anolyte was evaluated for *B. anthracis* and *B. subtilis* on six building material surfaces. The decontamination efficacy of 3,500 ppm FAC, pH 5 anolyte (60 min contact time, two total spray applications) for *B. anthracis* was ≥ 7 log reduction on glass, equivalent to complete inactivation within the detection limit as shown in Table 9-1 and summarized in Table 9-3. The highest efficacy occurred on glass (≥ 7.62), a lower efficacy on decorative laminate (4.88), and near-complete inactivation was observed on galvanized metal (7.60), all non-porous materials. Lower efficacies occurred with industrial carpet (0.30), painted wallboard paper (2.43), and bare pine wood (0.81), all porous materials.

Similar results were seen for *B. subtilis*, as shown in Table 9-2 and summarized in Table 9-3, with the highest efficacy occurring on glass (≥ 7.71), a relatively high efficacy on decorative laminate (6.12), and near-complete inactivation observed on galvanized metal (7.06), all non-porous materials. Lower efficacies occurred with industrial carpet (0.65), painted wallboard paper (2.56), and bare pine wood (0.54), all porous materials.

Table 9-1. Inactivation of *Bacillus anthracis* spores—3,500 ppm FAC, pH 5 anolyte, by material (60 minute contact with re-application at 30 minutes for two total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.39×10^8	8.07 ± 0.03	84.46 ± 5.69	-
Test Coupons[c]	1.39×10^8	7.77 ± 0.09	43.43 ± 8.87	0.30 ± 0.08
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.39×10^8	7.93 ± 0.05	61.87 ± 7.74	-
Test Coupons	1.39×10^8	3.05 ± 2.34	0.067 ± 0.11	4.88 ± 2.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.39×10^8	7.91 ± 0.04	58.09 ± 5.26	-
Test Coupons	1.39×10^8	0.31 ± 0.69	< 0.01	7.60 ± 0.60
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.39×10^8	7.82 ± 0.15	49.70 ± 14.79	-
Test Coupons	1.39×10^8	5.39 ± 0.49	0.31 ± 0.41	2.43± 0.45
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.39×10^8	7.06 ± 0.12	8.48 ± 2.10	-
Test Coupons	1.39×10^8	6.25 ± 0.13	1.34 ± 0.39	0.81 ± 0.15
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.39×10^8	7.93 ± 0.09	62.48 ± 13.46	-
Test Coupons	1.39×10^8	0.31 ± 0.69	< 0.01	7.62 ± 0.60
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 9-2. Inactivation of *Bacillus subtilis* spores—3,500 ppm FAC, pH 5 anolyte, by material (60 minute contact with re-application at 30 minutes for two total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	9.83×10^7	7.82 ± 0.07	67.95 ± 11.20	-
Test Coupons[c]	9.83×10^7	7.17 ± 0.11	15.30 ± 3.93	0.65 ± 0.12
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	9.83×10^7	7.70 ± 0.20	55.70 ± 23.85	-
Test Coupons	9.83×10^7	1.58 ± 0.96	< 0.01	6.12 ± 0.86
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	9.83×10^7	7.77 ± 0.11	60.95 ± 14.63	-
Test Coupons	9.83×10^7	0.71 ± 0.99	< 0.01	7.06 ± 0.87
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	9.83×10^7	7.08 ± 0.07	12.30 ± 1.92	-
Test Coupons	9.83×10^7	4.52 ± 0.54	0.049 ± 0.034	2.56 ± 0.47
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	9.83×10^7	6.56 ± 0.33	4.35 ± 2.22	-
Test Coupons	9.83×10^7	6.02 ± 0.37	1.43 ± 1.17	0.54 ± 0.43
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	9.83×10^7	7.71 ± 0.22	57.73 ± 29.59	-
Test Coupons	9.83×10^7	0	0	≥7.71 ± 0.19
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 9-3. Summary of mean efficacy (log reduction) values for 3,500 ppm FAC, pH 5 anolyte (60 minute contact with re-application of spray at +30 minutes for two total applications)

Test Material	Efficacy for *B. anthracis* (Ames)	Efficacy for *B. subtilis*
Industrial Carpet	0.30	0.65
Decorative Laminate	4.88	6.12
Galvanized Metal	7.60	7.06
Painted Wallboard Paper	2.43	2.56
Bare Pine Wood	0.81	0.54
Glass	7.62[a]	7.71[a]

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/material.

9.3 Damage to Coupons

No visible damage was observed on the test materials after the 60 min contact time and two total spray applications with this anolyte (3500 ppm FAC, pH 5).

9.4 Other Factors

9.4.1 Anolyte Useful-Life

The measurements listed in Table 9-4 and graphed in Figure 9-1 show an FAC useful-life of greater than 90% and a pH useful-life of greater than 100% (pH increased over time) from the readings made at the time of anolyte generation (Time 0).

Table 9-4. Measurements and useful-life of 3,500 ppm FAC, pH 5 anolyte solution

Flow Rate (Lph)	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (mS)	Target FAC[1], pH	FAC, ORP[2], pH at Time 0	FAC, ORP, pH at +1 hr	FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
68.2	105	8.9	98	47.1	3500, 5	3670, 1060, 5.09	3490, 1073, 5.09	3328, 1082, 5.11	28.4

[1] Reported as ppm.
[2] Reported as mV.

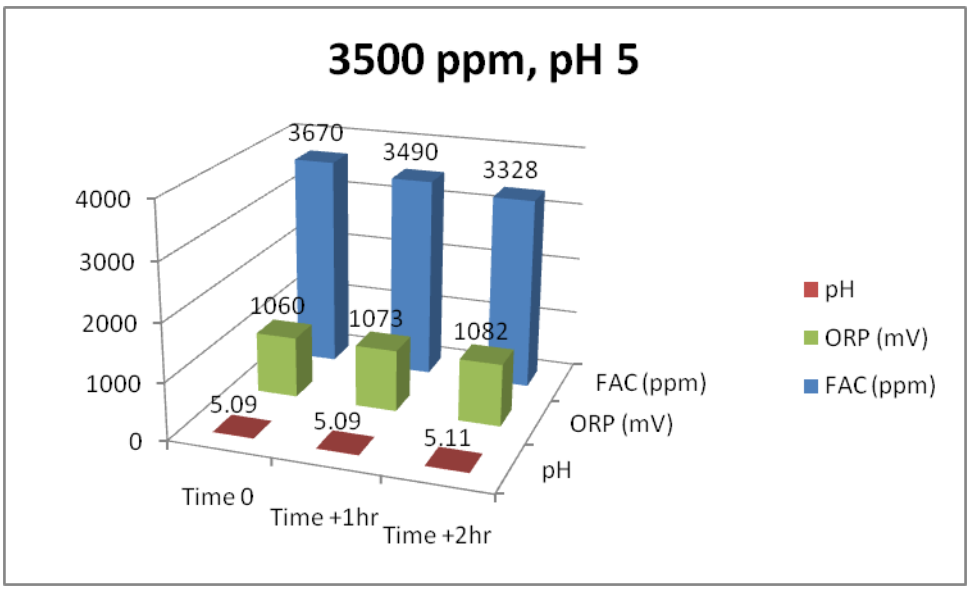


Figure 9-1. Measurements and useful-life for 3,500 ppm FAC, pH 5 anolyte

9.4.2 Anolyte Spray Deposition

The anolyte was applied from a distance of 30.5 cm (12 inches) to the horizontally-oriented materials until the materials were fully wetted. Re-application of the anolyte was made on all coupon surfaces at 30 minutes after the initial application, for a total of two spray applications. At 60 minutes after the initial application, each material coupon was placed in the 50 mL conical tube that also served to collect excess anolyte runoff.

Prior to decontamination testing, to assess the amount of anolyte deposited via spraying, triplicate coupons of each test material were weighed prior to application of the anolyte in the trial runs, and these values were recorded. Then the triplicate coupons were sprayed with anolyte until fully wetted in their horizontal orientations, re-application made at 30 minutes contact time for a total of two applications, and after 60 minutes contact time each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the anolyte from each of the test materials is shown in Table 9-5. The total averaged value (0.28 g) over all six materials was then used to estimate the amount of STS needed to neutralize the anolyte effectively under this testing condition.

Table 9-5. Deposition/runoff weight of 3,500 ppm FAC, pH 5 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Test Material	Average Deposition/Runoff Weight (g)
Industrial Carpet	0.25
Decorative Laminate	0.18
Galvanized Metal	0.30
Painted Wallboard Paper	0.32
Bare Pine Wood	0.32
Glass	0.32
Average	*0.28*

9.4.3 Neutralization Methodology

Neutralization of the 3,500 ppm FAC, pH 5 anolyte was achieved with STS. The concentrations of STS tried during the neutralization panels were 0.5, 1.0, 1.5, and 2.0% in the extraction solution. The neutralization range was expanded from previous trials since the FAC concentration jumped from 3,000 to 3,500 ppm, so the expanded range was simply an attempt to effectively neutralize the FAC with a single attempt. The results of the neutralization panels are shown in Table 9-6 and 9-7. From these panels, 1.0% STS was determined to be sufficient for neutralization of the 3500 ppm FAC, pH 5 anolyte for *B. anthracis* and 2.0% STS for *B. subtilis*.

Table 9-6. Neutralization testing with *Bacillus anthracis* spores with 3,500 ppm FAC, pH 5 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	1.16×10^8	0	0
Anolyte + PBS + Triton X-100 + Spores[ab]	1.16×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.16×10^8	1.12×10^8	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	1.16×10^8	1.04×10^8	92.52
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	1.16×10^8	1.11×10^8	104.33
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	1.16×10^8	1.17×10^8	104.33
Anolyte + PBS + Triton X-100 + 2.0% STS + Spores[ab]	1.16×10^8	1.12×10^8	99.44

[a] Anolyte volume of 0.28 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.

[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.28 mL (10 mL PBS/Triton X-100/STS + 0.28 mL anolyte).

Table 9-7. Neutralization testing with *Bacillus subtilis* spores with 3,500 ppm FAC, pH 5 anolyte (60 minute contact with re-application at +30 minutes for two total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	9.90×10^7	0	0
Anolyte + PBS + Triton X-100 + Spores[ab]	9.90×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	9.90×10^7	1.12×10^8	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	9.90×10^7	1.26×10^8	112.93
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	9.90×10^7	1.25×10^8	111.70
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	9.90×10^7	1.20×10^8	107.71
Anolyte + PBS + Triton X-100 + 2.0% STS + Spores[ab]	9.90×10^7	1.26×10^8	112.93

[a] Anolyte volume of 0.28 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.

[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.28 mL (10 mL PBS/Triton X-100/STS + 0.28 mL anolyte).

10.0 Anolyte Solution Test Results for 3,500 ppm FAC, pH 5, 120 Minute Contact

10.1 QC Results

The anolyte solution with a target of 3,500 ppm FAC, pH 5 was sprayed at Time 0, +30, +60, and at +90 minutes for a total of four spray applications with a total contact time of 120 minutes (i.e., anolyte allowed to dwell for an additional 30 minutes after the +90 minute spray). In testing of this anolyte, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values for *B. anthracis* spores ranged from 3.76 to 79.07%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on industrial carpet. Positive control recovery values for *B. subtilis* spores ranged from 2.79 to 46.87%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on industrial carpet. Refer to Tables 10-1 and 10-2.

In testing of the 3,500 ppm FAC, pH 5 anolyte (120 min contact time, four total spray applications), all procedural and laboratory blanks met the criterion of no observed CFU.

Spike control samples were taken from the spore suspension on each day of testing and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for *B. anthracis* and *B. subtilis* testing for this anolyte batch were 1.17×10^8/coupon and 1.10×10^8/coupon, respectively.

10.2 Decontamination Efficacy

The decontamination efficacy of 3,500 ppm FAC, pH 5 anolyte was evaluated for *B. anthracis* and *B. subtilis* on six building material surfaces. The decontamination efficacy of 3,500 ppm, pH 5 anolyte (120 min contact time, four total spray applications) for *B. anthracis* was ≥ 7 log reduction on three of six materials. The efficacy results were equivalent to complete inactivation within the detection limit as shown in Table 10-1 and summarized in Table 10-3. The highest efficacies occurred on decorative laminate (≥ 7.50), galvanized metal (≥ 7.81), and glass (≥ 7.87), all non-porous materials. Lower efficacies occurred with industrial carpet (0.60), painted wallboard paper (2.37), and bare pine wood (0.89), all porous materials.

Similar results were seen for *B. subtilis*, as shown in Table 10-2 and summarized in Table 10-3, with ≥ 7 log reduction on three of six materials. The efficacy results were equivalent to complete inactivation within the detectable limit on decorative laminate (≥ 7.62), galvanized metal (≥ 7.60), and glass (≥ 7.66), all non-porous materials. Lower efficacies occurred with industrial carpet (0.97), painted wallboard paper (2.44), and bare pine wood (0.76).

Table 10-1. Inactivation of *Bacillus anthracis* spores—3,500 ppm FAC, pH 5 anolyte, by material (120 minute contact with re-applications at 30, 60, and 90 minutes for four total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.17×10^8	7.96 ± 0.04	79.07 ± 7.19	-
Test Coupons[c]	1.17×10^8	7.36 ± 0.21	21.21 ± 9.55	0.60 ± 0.18
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.17×10^8	7.50 ± 0.26	30.25 ± 13.46	-
Test Coupons	1.17×10^8	0	0	≥7.50 ± 0.23
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.17×10^8	7.81 ± 0.04	54.93 ± 5.31	-
Test Coupons	1.17×10^8	0	0	≥7.81 ± 0.04
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.17×10^8	7.66 ± 0.10	39.80 ± 8.37	-
Test Coupons	1.17×10^8	5.29 ± 0.80	0.46 ± 0.54	2.37 ± 0.70
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.17×10^8	6.60 ± 0.23	3.76 ± 1.63	-
Test Coupons	1.17×10^8	5.71 ± 0.36	0.53 ± 0.31	0.89 ± 0.37
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.17×10^8	7.87 ± 0.02	62.77 ± 2.98	-
Test Coupons	1.17×10^8	0	0	≥7.87 ± 0.02
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 10-2. Inactivation of *Bacillus subtilis* spores—3,500 ppm FAC, pH 5 anolyte, by material (120 minute contact with re-applications at 30, 60, and 90 minutes for four total spray applications)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.10×10^8	7.71 ± 0.08	46.87 ± 8.92	-
Test Coupons[c]	1.10×10^8	6.74 ± 0.17	5.24 ± 1.67	0.97 ± 0.16
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.10×10^8	7.62 ± 0.03	38.38 ± 2.36	-
Test Coupons	1.10×10^8	0	0	≥7.62 ± 0.02
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.10×10^8	7.60 ± 0.05	36.57 ± 4.54	-
Test Coupons	1.10×10^8	0	0	≥7.60 ± 0.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.10×10^8	7.19 ± 0.19	15.27 ± 6.64	-
Test Coupons	1.10×10^8	4.75 ± 0.60	0.10 ± 0.12	2.44 ± 0.55
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.10×10^8	6.42 ± 0.29	2.79 ± 1.57	-
Test Coupons	1.10×10^8	5.66 ± 0.49	0.66 ± 0.72	0.76 ± 0.50
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.10×10^8	7.66 ± 0.10	42.22 ± 9.08	-
Test Coupons	1.10×10^8	0	0	≥7.66 ± 0.08
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 10-3. Summary of mean efficacy (log reduction) values for 3,500 ppm FAC, pH 5 anolyte (120 minute contact with re-application of sprays at 30, 60, and 90 minutes for four total applications)

Test Material	Efficacy for *B. anthracis* (Ames)	Efficacy for *B. subtilis*
Industrial Carpet	0.60	0.97
Decorative Laminate	7.50[a]	7.62[a]
Galvanized Metal	7.81[a]	7.60[a]
Painted Wallboard Paper	2.37	2.44
Bare Pine Wood	0.89	0.76
Glass	7.87[a]	7.66[a]

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/material.

10.3 Damage to Coupons

No visible damage was observed on the test materials after the 120 min contact time and four total spray applications with this anolyte (3,500 ppm FAC, pH 5).

10.4 Other Factors

10.4.1 Anolyte Useful-Life

The measurements listed in Table 10-4 and graphed in Figure 10-1 show an FAC useful-life of greater than 97% and a pH useful-life of greater than 97% from the readings made at the time of anolyte generation (Time 0).

Table 10-4. Measurements and useful-life of 3,500 ppm FAC, pH 5 anolyte solution

Flow Rate (Lph)	Power Input (A)	Power Input (V)	Brine Pump Speed (%)	Brine Solution Conductivity (mS)	Target FAC[1], pH	FAC, ORP[2], pH at Time 0	FAC, ORP, pH at +1 hr	FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
68.2	105	8.90	100	47.1	3500, 5	3798, 1075, 5.08	3780, 1078, 4.94	3685, 1094, 4.95	28.4

[1] Reported as ppm.
[2] Reported as mV.

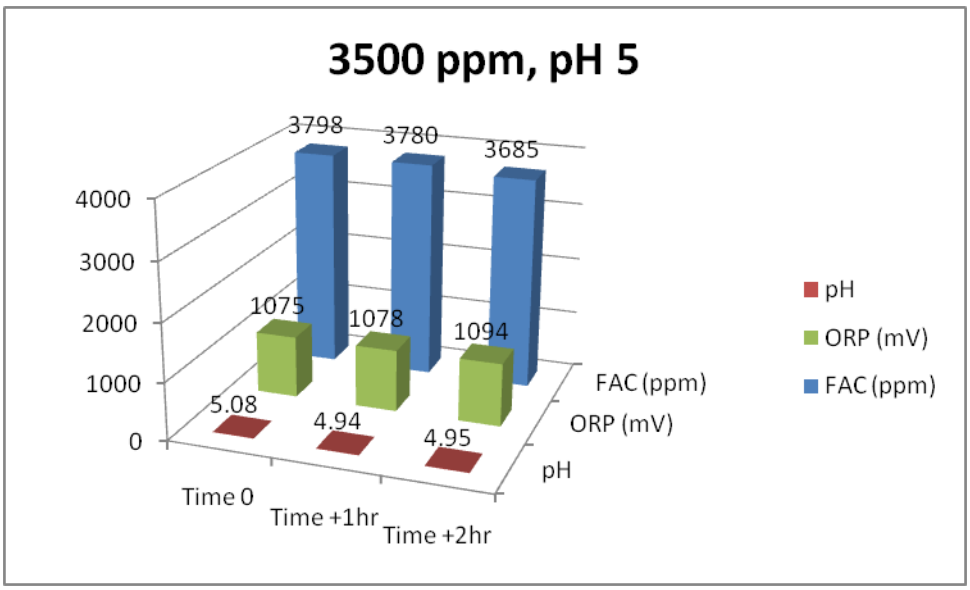


Figure 10-1. Measurements and useful-life for 3,500 ppm FAC, pH 5 anolyte (120 minute contact)

10.4.2 Anolyte Spray Deposition

The anolyte was applied from a distance of 30.5 cm (12 inches) to the horizontally-oriented materials until the materials were fully wetted. Re-application of the anolyte was made on all coupon surfaces at 30, 60, and 90 minutes after the initial application, for a total of four total spray applications. At 120 minutes after the initial application, each material coupon was placed in the 50 mL conical tube that also served to collect excess anolyte runoff. The test coupons stayed in their horizontal orientation throughout the 120 minute contact time.

Prior to decontamination testing, to assess the amount of anolyte deposited via spraying, triplicate coupons of each test material were weighed prior to application of the anolyte in the trial runs, and these values were recorded. Then the triplicate coupons were sprayed with anolyte until fully wetted in their horizontal orientations. Re-applications were made at 30, 60, and 90 minutes contact times for a total of four applications. After 120 minutes contact time, each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the anolyte from each of the test materials is shown in Table 10-5. The total averaged value (0.57 g) over all six materials was then used to estimate the amount of STS needed to neutralize the anolyte effectively under this testing condition.

Table 10-5. Deposition/runoff weight of 3,500 ppm FAC, pH 5 anolyte (120 minute contact with re-applications at 30, 60, and 90 minutes for four total spray applications)

Test Material	Average Deposition/Runoff Weight (g)
Industrial Carpet	0.75
Decorative Laminate	0.56
Galvanized Metal	0.43
Painted Wallboard Paper	0.42
Bare Pine Wood	0.65
Glass	0.58
Average	*0.57*

10.4.3 Neutralization Methodology

Neutralization of the 3,500 ppm FAC, pH 5 anolyte was achieved with STS. The concentrations of STS tried during the neutralization panels were 0.5, 1.0, 1.5, and 2.0% in the extraction solution. The neutralization range was expanded from previous panels since the FAC concentration not only jumped from 3,000 to 3,500 ppm but also the contact time doubled from 60 to 120 minutes, so the expanded range was simply an attempt to effectively neutralize the FAC with a single attempt. The results of the neutralization panels are shown in Table 10-6 and 10-7. From these panels, 0.5% STS was determined to be sufficient for neutralization of the 3,500 ppm FAC, pH 5, anolyte for *B. anthracis* and 2.0% STS for *B. subtilis*.

Table 10-6. Neutralization testing with *Bacillus anthracis* spores with 3,500 ppm FAC, pH 5 anolyte (120 minute contact with re-application at 30, 60, and 90 minutes for four total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	1.21×10^8	0	0
Anolyte + PBS + Triton X-100 + Spores[ab]	1.21×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.21×10^8	1.02×10^8	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	1.21×10^8	1.12×10^8	109.50
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	1.21×10^8	1.10×10^8	108.22
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	1.21×10^8	1.01×10^8	98.69
Anolyte + PBS + Triton X-100 + 2.0% STS + Spores[ab]	1.21×10^8	1.05×10^8	102.94

[a] Anolyte volume of 0.57 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.

[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.57 mL (10 mL PBS/Triton X-100/STS + 0.57 mL anolyte).

Table 10-7. Neutralization testing with *Bacillus subtilis* spores with 3,500 ppm FAC, pH 5 anolyte (120 minute contact with re-application at 30, 60, and 90 minutes for four total spray applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Anolyte + Spores[a]	1.28×10^8	0	0
Anolyte + PBS + Triton X-100 + Spores[ab]	1.28×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.28×10^8	1.14×10^8	100
Anolyte + PBS + Triton X-100 + 0.5% STS + Spores[ab]	1.28×10^8	1.04×10^8	91.53
Anolyte + PBS + Triton X-100 + 1.0% STS + Spores[ab]	1.28×10^8	1.01×10^8	89.18
Anolyte + PBS + Triton X-100 + 1.5% STS + Spores[ab]	1.28×10^8	1.09×10^8	96.09
Anolyte + PBS + Triton X-100 + 2.0% STS + Spores[ab]	1.28×10^8	1.11×10^8	97.95

[a] Anolyte volume of 0.57 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.

[b] 10 mL volume of PBS includes 0.1% of Triton X-100 surfactant and indicated % of STS; total volume for all samples with anolyte = 10.57 mL (10 mL PBS/Triton X-100/STS + 0.57 mL anolyte).

11.0 Anolyte Solution Test Results for 3,500 ppm FAC, pH 5, 18 Hour Contact

11.1 QC Results

The anolyte solution with a target of 3,500 ppm FAC, pH 5 was sprayed at Time 0, +30, +60, and at +90 minutes for a total of four spray applications with a total contact time of 120 minutes (i.e., anolyte allowed to dwell for an additional 30 minutes after the +90 minute spray), but then the sprayed materials were allowed to sit undisturbed overnight (18 hours). This process was applied to the positive controls sprayed with SFW. The following day, the materials were processed in the typical fashion. Refer to Tables 11-1 and 11-2.

In testing of this anolyte, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values for *B. anthracis* spores ranged from 12.92 to 93.47%, with the lowest recovery occurring on bare pine wood and the highest recovery occurring on industrial carpet. Positive control recovery values for *B. subtilis* spores ranged from 2.59 to 55.22%, with the lowest recovery occurring on bare pine wood, and the highest recovery occurring on industrial carpet.

In testing of the 3,500 ppm FAC, pH 5 anolyte (overnight contact time, four total spray applications), all procedural and laboratory blanks met the criterion of no observed CFU.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for *B. anthracis* and *B. subtilis* testing for this anolyte batch were 8.00×10^7/coupon and 1.40×10^8/coupon, respectively.

11.2 Decontamination Efficacy

The decontamination efficacy of 3,500 ppm FAC, pH 5 anolyte was evaluated for *B. anthracis* and *B. subtilis* on six building material surfaces. The decontamination efficacy of 3,500 ppm, pH 5 anolyte (overnight contact time, four total spray applications) for *B. anthracis* was ≥ 7 log reduction on three of six materials; the efficacy was equivalent to complete inactivation within the detection limit as shown in Table 11-1 and summarized in Table 11-3. The highest efficacies occurred on decorative laminate (≥ 7.54), galvanized metal (≥ 7.68), and glass (≥ 7.73), all non-porous materials. Lower efficacies occurred with industrial carpet (0.45), painted wallboard paper (3.47), and bare pine wood (0.97), all porous materials.

Similar results were seen for *B. subtilis*, as shown in Table 11-2 and summarized in Table 11-3, with ≥ 7 log reduction on three of six materials. The efficacies were equivalent to complete inactivation within the detection limit on decorative laminate (≥

7.54), galvanized metal (≥ 7.60), and glass (≥ 7.60), all non-porous materials. Lower efficacies occurred with industrial carpet (0.84), painted wallboard paper (3.45), and bare pine wood (0.67).

Table 11-1. Inactivation of *Bacillus anthracis* spores—3,500 ppm FAC, pH 5 anolyte, by material (120 minute contact with re-applications at 30, 60, and 90 minutes for four total spray applications, 18 hour total contact)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	8.00×10^7	7.87 ± 0.02	93.47 ± 4.04	-
Test Coupons[c]	8.00×10^7	7.42 ± 0.20	35.44 ± 16.67	0.46 ± 0.17
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	8.00×10^7	7.60 ± 0.05	49.68 ± 6.07	-
Test Coupons	8.00×10^7	0	0	≥7.60 ± 0.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	8.00×10^7	7.68 ± 0.12	61.04 ± 16.39	-
Test Coupons	8.00×10^7	0	0	≥7.68 ± 0.10
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	8.00×10^7	7.52 ± 0.06	41.49 ± 5.69	-
Test Coupons	8.00×10^7	4.05 ± 0.29	0.016 ± 0.01	3.47 ± 0.26
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	8.00×10^7	6.96 ± 0.25	12.92 ± 6.73	-
Test Coupons	8.00×10^7	5.99 ± 0.40	1.53 ± 0.81	0.97 ± 0.42
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	8.00×10^7	7.73 ± 0.15	70.18 ± 22.13	-
Test Coupons	8.00×10^7	0	0	≥7.73 ± 0.13
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = inoculated, decontaminated coupons.
[d] Laboratory Blank = not inoculated, not decontaminated coupon.
[e] Procedural Blank = not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 11-2. Inactivation of *Bacillus subtilis* spores—3,500 ppm FAC, pH 5 anolyte, by material (120 minute contact with re-applications at 30, 60, and 90 minutes for four total spray applications, 18 hour total contact)[a]

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Industrial Carpet				
Positive Controls[b]	1.40×10^8	7.89 ± 0.03	55.22 ± 3.52	-
Test Coupons[c]	1.40×10^8	7.05 ± 0.10	8.15 ± 2.00	0.84 ± 0.09
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Decorative Laminate				
Positive Controls	1.40×10^8	7.54 ± 0.08	21.17 ± 5.10	-
Test Coupons	1.40×10^8	0	0	≥7.54 ± 0.07
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Galvanized Metal				
Positive Controls	1.40×10^8	7.60 ± 0.05	28.24 ± 2.90	-
Test Coupons	1.40×10^8	0	0	≥7.60 ± 0.04
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Painted Wallboard Paper				
Positive Controls	1.40×10^8	7.06 ± 0.12	8.52 ± 2.56	-
Test Coupons	1.40×10^8	3.61 ± 0.72	< 0.01	3.45 ± 0.64
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Bare Pine Wood				
Positive Controls	1.40×10^8	6.51 ± 0.26	2.59 ± 1.16	-
Test Coupons	1.40×10^8	5.84 ± 0.36	0.63 ± 0.38	0.66 ± 0.39
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Glass				
Positive Controls	1.40×10^8	7.60 ± 0.04	28.49 ± 2.67	-
Test Coupons	1.40×10^8	0	0	≥7.60 ± 0.04
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = confidence interval (± 1.96 × SE).

[b] Positive Controls = inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = inoculated, decontaminated coupons.

[d] Laboratory Blank = not inoculated, not decontaminated coupon.

[e] Procedural Blank = not inoculated, decontaminated coupon.

"-" Not Applicable.

Table 11-3. Summary of mean efficacy (log reduction) values for 3,500 ppm, pH 5 anolyte (120 minute contact with re-application of sprays at 30, 60, and 90 minutes for four total applications, 18 hour total contact)

Test Material	Efficacy for *B. anthracis* (Ames)	Efficacy for *B. subtilis*
Industrial Carpet	0.46	0.84
Decorative Laminate	7.60[a]	7.54[a]
Galvanized Metal	7.68[a]	7.60[a]
Painted Wallboard Paper	3.47	3.45
Bare Pine Wood	0.97	0.66
Glass	7.73[a]	7.60[a]

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/material.

11.3 Damage to Coupons

No visible damage was observed on the test materials after the overnight contact time and four total spray applications with this anolyte (3,500 ppm FAC, pH 5).

11.4 Other Factors

11.4.1 Anolyte Useful-Life

The measurements listed in Table 11-4 and graphed in Figure 11-1 show an FAC useful-life of greater than 96% and a pH useful-life of greater than 99% from the readings made at the time of anolyte generation (Time 0).

Table 11-4. Measurements and useful-life of 3,500 ppm FAC, pH 5 anolyte solution

Flow Rate (Lph)	Power Input (amps)	Power Input (volts)	Brine Pump Speed (%)	Brine Solution Conductivity (mS)	Target FAC[1], pH	FAC, ORP[2], pH at Time 0	FAC, ORP, pH at +1 hr	FAC, ORP, pH at +2 hr	Anolyte Production Rate (Lph)
64.4	108	8.60	98	47.1	3500, 5	3465, 1090, 5.03	3375, 1098, 4.99	3355, 1097, 4.99	28.4

[1] Reported as ppm.
[2] Reported as mV.

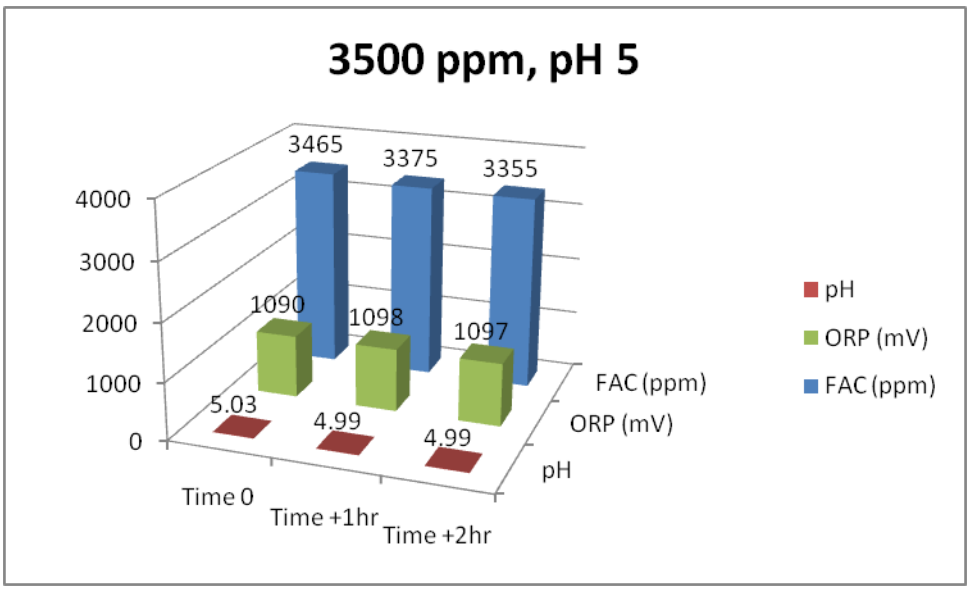


Figure 11-1. Measurements and useful-life for 3,500 ppm FAC, pH 5 anolyte (18 hour total contact)

11.4.2 Anolyte Spray Deposition

The anolyte was applied from a distance of 30.5 cm (12 inches) to the horizontally-oriented materials until the materials were fully wetted. Re-application of the anolyte was made on all coupon surfaces at 30, 60, and 90 minutes after the initial application, for a spray total of four applications. At 120 minutes after the initial application, each material coupon was allowed to dwell overnight in the glovebox. The next morning after the 18 hour total contact time, each material coupon was placed in the 50 mL conical tube for extraction.

For this test, no assessment of anolyte deposition was done since it would have been the same process as that described in Chapter 9 (Section 9.4.2). The only change that occurred in this test was the incorporation of an overnight contact time after the initial 120 minute contact time and four total applications. The average deposition/runoff weight of the anolyte from each of the test materials is shown in Table 9-5. The total averaged value (0.57 g) over all six materials was then used to estimate the amount of STS needed to neutralize the anolyte effectively under this testing condition.

11.4.3 Neutralization Methodology

Neutralization of the 3,500 ppm FAC, pH 5 anolyte was achieved with STS and was the same as that described in Chapter 10 (Section 10.4.3) for the same reason that the spray deposition from Chapter 9 was used. The results of the neutralization panels are shown in Tables 10-6 and 10-7. From these trials, 0.5% STS was determined to be sufficient for neutralization of the 3,500 ppm FAC, pH 5, anolyte for *B. anthracis* and 2.0% STS for *B. subtilis*.

12.0 Summary

The quantitative decontamination efficacies for *B. anthracis* and *B. subtilis* were ≥ 4.55 log reduction on all non-porous materials (decorative laminate, galvanized metal, and glass; refer to Figure 12-1 and Tables 12-1 and 12-2) for all anolyte solutions, contact times, and application rates tested. These materials were the only ones that were completely decontaminated in at least one test condition.

The quantitative efficacies for the porous materials (industrial carpet, painted wallboard paper, and bare pine wood; refer to Figure 12-2) were all ≤ 3.47 log reduction. Except for one test, all of the mean log reduction results for industrial carpet and bare pine wood were ≤ 1.

The decontamination efficacies (log reduction) for *B. subtilis* are compared to *B. anthracis* in Table 12-3. Out of 36 test conditions, there were 11 in which the results for the two microorganisms were significantly different. Differences were determined to be significant if the 95% confidence intervals for the log reduction results for the two microorganisms did not overlap. We note that there were a few test conditions in which both *B. anthracis* and *B. subtilis* were completely inactivated and the log reduction results were determined to be significantly different for the two microorganisms. (See for example the results for galvanized metal, 3500 ppm FAC, 120 minute contact time.) In these cases, the difference in log reduction results may be due to differences in inoculum level or recovery efficiency.

Changing pH, number of spray applications, contact time, or FAC level did not significantly affect (based on whether the 95% confidence intervals for the log reduction results overlapped or not) decontamination efficacy results except for a few instances. Significant differences in results occurred for the non-porous materials (*B. anthracis* or *B. subtilis*) essentially only when tests at 3000 ppm FAC, 1 hour contact time, changed from pH 5 to pH 6. Further changes in test conditions had minimal effect on the non-porous materials, since nearly all log reduction results were greater than 7. For the porous materials, there were only a few tests in which significant improvements in decontamination efficacy were achieved by varying pH, number of spray applications, contact time, or FAC level.

The useful-life evaluations for each test anolyte (in terms of pH, ORP, and FAC level) showed that only gradual degradation (less than 9% FAC loss) occurred over the two- hour span (Figures 5-1 to 5-4) for all anolyte solutions with the exception of one. (These elapsed times were chosen for testing because they were determined to be representative of the elapsed times these anolyte solutions would most likely be used in the field before their replacement.) Storing the anolyte in sealed, HDPE containers with minimal head-space, as IET had recommended, may have mitigated off-gassing of chlorine from the anolyte solutions, thus reducing degradation over the two- hour span.

The anolyte optimization tests showed that once the parameters and settings achieved targeted FAC levels of 1,000, 2,000, 3,000, and 3,500 ppm, each at pH levels of 5, 6, and 7 (3,500 ppm FAC required only pH 5), the EcaFlo® reliably reproduced and quickly generated anolyte at the desired FAC and pH levels with only slight adjustments required.

Table 12-1. Summary of Decontamination Results for *Bacillus anthracis*

Test Material	Quantitative Efficacy (mean log reduction)					
	3000 ppm FAC, pH 5, 60 min contact time, two total spray applications	3000 ppm FAC, pH 6, 60 min contact time, two total spray applications	3000 ppm FAC, pH 7, 60 min contact time, two total spray applications	3500 ppm FAC, pH 5, 60 min contact time, two total spray applications	3500 ppm FAC, pH 5, 120 min contact time, four total spray applications	3500 ppm FAC, pH 5,18 hr contact time, four total spray applications
Industrial Carpet	0.58	0.26	0.31	0.30	0.60	0.45
Decorative Laminate	5.95	7.55[a]	7.28[a]	4.88	7.50[a]	7.60[a]
Galvanized Metal	4.58	7.46	7.61	7.60	7.81[a]	7.68[a]
Painted Wallboard Paper	2.57	2.18	2.62	2.43	2.37	3.47
Bare Pine Wood	2.13	0.68	1.02	0.81	0.89	0.97
Glass	4.55	7.83[a]	7.93[a]	7.62[a]	7.87[a]	7.73[a]

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/material.

Table 12-2. Summary of Decontamination Results for *Bacillus subtilis*

Test Material	Quantitative Efficacy (mean log reduction)					
	3000 ppm FAC, pH 5, 60 min contact time, two total spray applications	3000 ppm FAC, pH 6, 60 min contact time, two total spray applications	3000 ppm FAC, pH 7, 60 min contact time, two total spray applications	3500 ppm FAC, pH 5, 60 min contact time, two total spray applications	3500 ppm FAC, pH 5, 120 min contact time, four total spray applications	3500 ppm FAC, pH 5,18 hr contact time, four total spray applications
Industrial Carpet	0.73	0.21	0.61	0.65	0.97	0.84
Decorative Laminate	5.95	7.57[a]	6.91[a]	6.12	7.62[a]	7.54[a]
Galvanized Metal	7.71[a]	7.79[a]	7.98[a]	7.06	7.60[a]	7.60[a]
Painted Wallboard Paper	1.71	2.51	3.01	2.56	2.44	3.45
Bare Pine Wood	0.30	0.47	0.67	0.54	0.76	0.67
Glass	6.14	7.75[a]	7.85[a]	7.71[a]	7.66[a]	7.60[a]

[a]Result represents complete inactivation within the detection limit of 33.33 CFU/material.

Table 12-3. Comparing efficacy (log reduction) between *B. anthracis* vs. *B. subtilis* by testing condition[b]

Test Material	Quantitative Efficacy (± 95% CI)											
	3000 ppm FAC, pH 5, 60 min contact time, two total spray applications		3000 ppm FAC, pH 6, 60 min contact time, two total spray applications		3000 ppm FAC, pH 7, 60 min contact time, two total spray applications		3500 ppm FAC, pH 5, 60 min contact time, two total spray applications		3500 ppm FAC, pH 5, 120 min contact time, four total spray applications		3500 ppm FAC, pH 5, 18 hr contact time, four total spray applications	
	B.a.	*B.s.*	*B.a.*	*B.s.*	*B.a.*	*B.s.*	*B.a.*	*B.s.*	*B.a.*	*B.s.*	*B.a.*	*B.s.*
Industrial Carpet	0.58 (±0.24)	**0.73[b]** (±0.07)	0.26 (±0.08)	0.21 (±0.06)	0.30 (±0.06)	**0.60[b]** (±0.13)	0.30 (±0.08)	**0.65[b]** (±0.12)	0.61 (±0.18)	**0.97[b]** (±0.09)	0.46 (±0.17)	**0.84[b]** (±0.09)
Decorative Laminate	5.94 (±1.05)	5.96 (±0.88)	7.55[a] (±0.23)	7.57[a] (±0.06)	7.28[a] (±0.74)	6.91[a] (±0.16)	4.88 (±2.05)	6.12 (±0.86)	7.50[a] (±0.23)	7.62[a] (±0.02)	7.60[a] (±0.05)	7.54[a] (±0.07)
Galvanized Metal	4.58 (±0.12)	**7.71[ab]** (±0.07)	7.46 (±0.72)	7.79[a] (±0.04)	7.61 (±0.60)	7.98[a] (±0.05)	7.60 (±0.60)	7.06 (±0.87)	7.81[a] (±0.04)	**7.60[ab]** (±0.05)	7.68[a] (±0.10)	7.60[a] (±0.04)
Painted Wallboard Paper	2.57 (±0.18)	**1.71[b]** (±0.65)	2.19 (±0.30)	2.51 (±0.25)	2.62 (±0.64)	3.01 (±0.08)	2.43 (±0.45)	2.56 (±0.47)	2.37 (±0.70)	2.45 (±0.55)	3.47 (±0.26)	3.45 (±0.64)
Bare Pine Wood	2.13 (±0.26)	**0.31[b]** (±0.14)	0.69 (±0.19)	0.47 (±0.09)	1.00 (±0.23)	0.67 (±0.25)	0.80 (±0.15)	0.54 (±0.43)	0.89 (±0.37)	0.76 (±0.50)	0.97 (±0.42)	0.66 (±0.39)
Glass	4.55 (±0.14)	**6.14[b]** (±1.05)	7.83[a] (±0.07)	7.75[a] (±0.04)	7.93[a] (±0.04)	7.85[a] (±0.11)	7.62[a] (±0.60)	7.71[a] (±0.19)	7.87[a] (±0.02)	**7.66[ab]** (±0.08)	7.73[a] (±0.13)	7.60[a] (±0.04)

[a] Result represents complete inactivation within the detection limit of 33.33 CFU/material.

[b] Values in bold for *B. subtilis* by testing condition are significantly different from corresponding values for *B. anthracis* if the 95% CIs of the two efficacy results did not overlap.

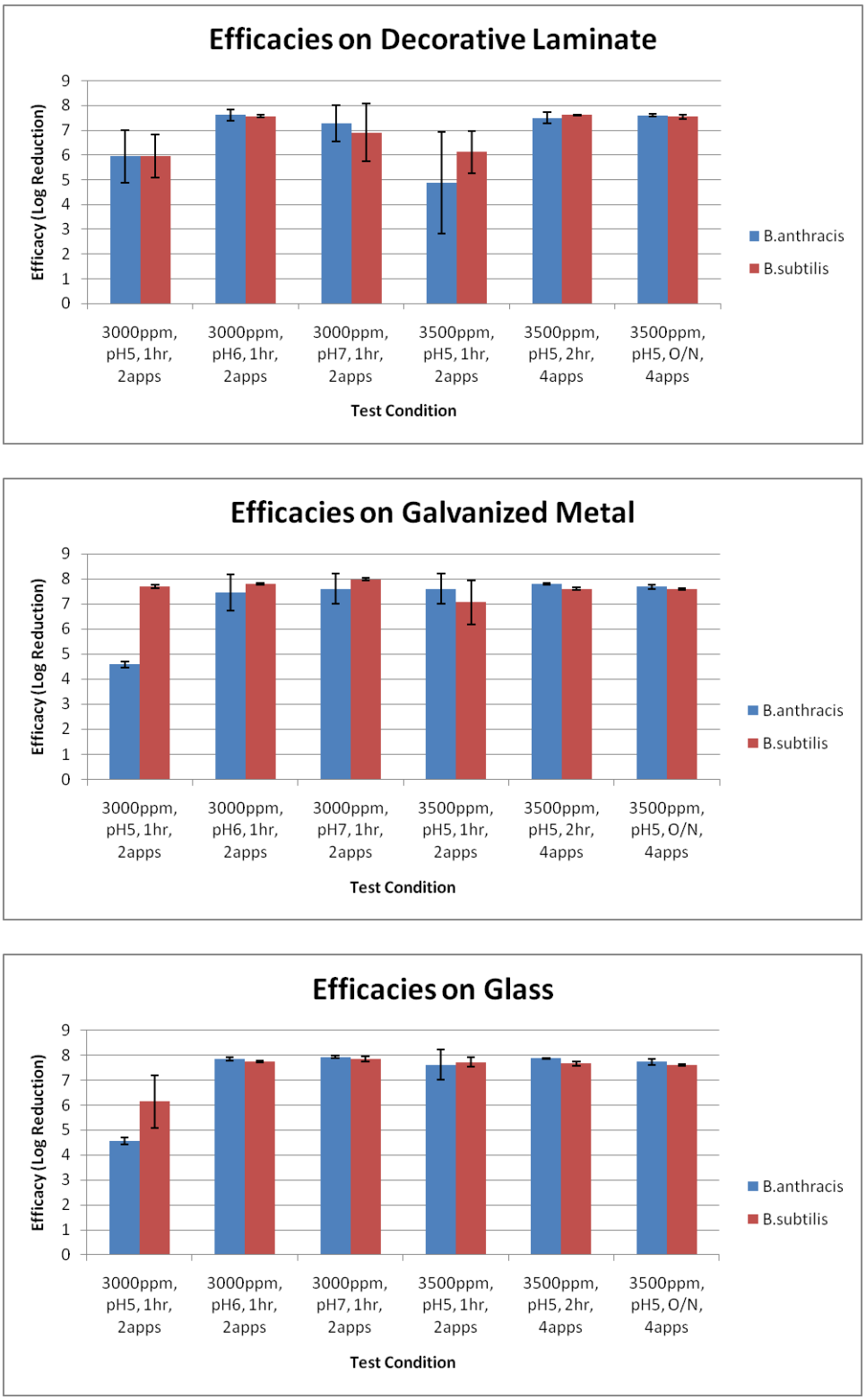
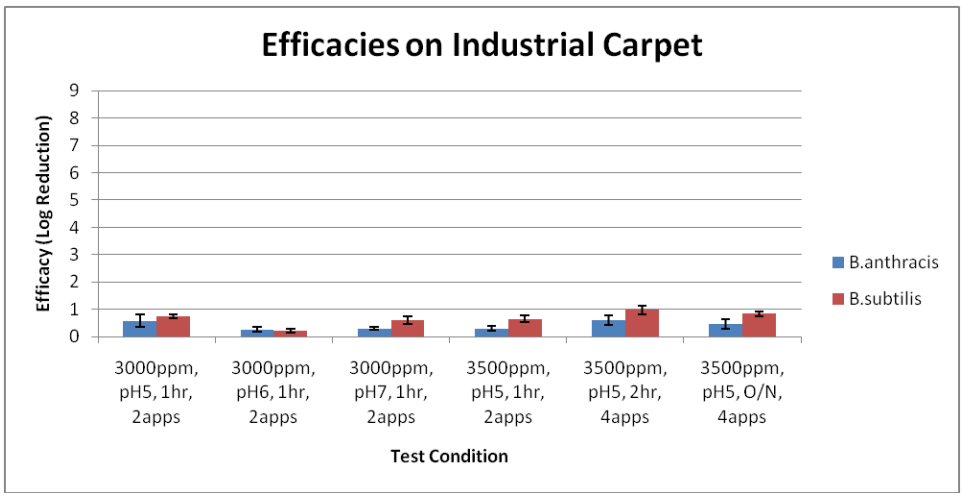
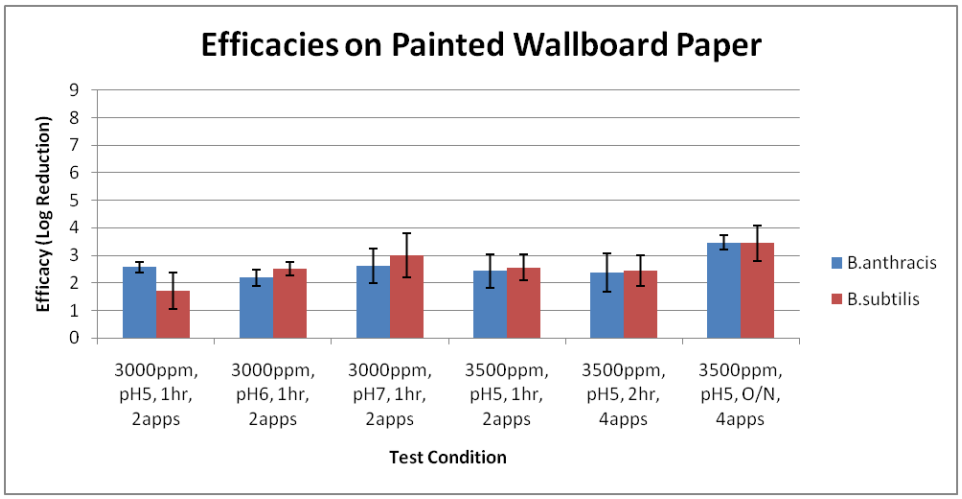


Figure 12-1. Quantitative decontamination efficacies (log reduction ± 95% CI) for the non-porous materials





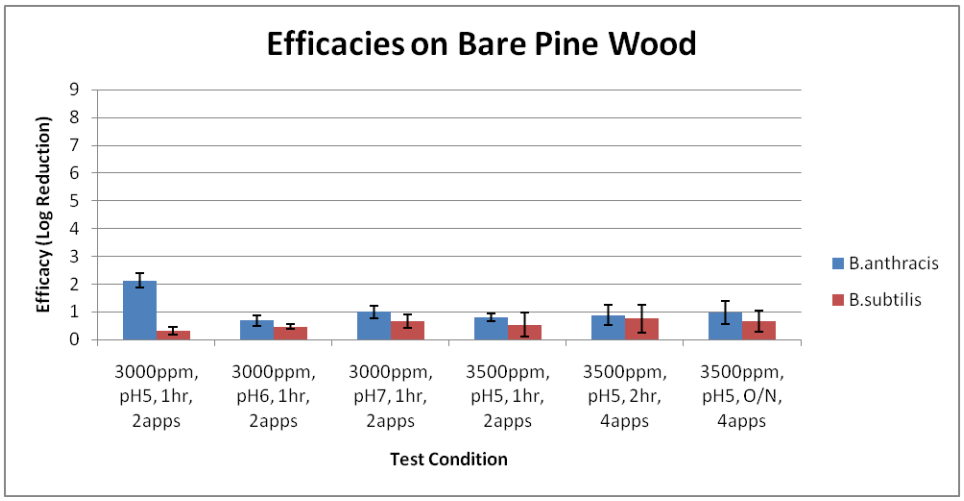


Figure 12-2. Quantitative decontamination efficacies (log reduction ± 95% CI) for the porous materials

12.0 References

1. Integrated Environmental Technologies website. http://www.ietecaflo.com/ Accessed September 30, 2011.

2. *Quality Management Plan, National Homeland Security Research Center, Office of Research and Development*, U.S. Environmental Protection Agency; Amendment to Solicitation PR-CI-09-10042, Cincinnati Procurement Operations Division, posted July 13, 2009, http://www.epa.gov/oamcinc1/09100 42/coverpg.htm, last accessed July 12, 2011.

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