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Case Study

Annette Baker^a

^a Fiberlock Technologies, Inc., Andover, Massachusetts

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Case Study

Quaternary Ammonium Compound (QAC): Case Study of Disinfectant Field Application Methodology and Effectiveness During Hurricane Katrina Flooding

INTRODUCTION AND BACKGROUND

This article describes a practical and economical method for disinfecting Category 3⁽¹⁾ contamination using Environmental Protection Agency (EPA)-registered quaternary ammonium active having 98% organic soil load and hard water efficacy. The cleaning protocol development and validation of disinfectant effectiveness under challenging application conditions is discussed.

Limited field trial information exists on Category 3 contamination and quaternary ammonium compound (QAC) efficacy under challenging application and environmental conditions. Therefore, a field trial was conducted to test effectiveness of QAC given environmental factors of high organic soil load and hard water level with elevated pressure and temperature spray application conditions. Hard water is generally defined as water containing over 100 mg/L or ppm of calcium carbonate.

Elevated environmental factors experienced in the field, such as 98% organic soil load, are not incorporated into the Association of Official Analytical Chemists (AOAC) Use-Dilution Test Method for Testing Disinfectants.⁽²⁾ Usage parameters are addressed in the Supplemental Recommendations for Efficacy Data Requirements⁽³⁾ and tested at the discretion of the manufacturer. Manufacturers generally utilize usage pattern parameters of 5% organic soil load and 200 or 400 ppm hard water when submitting efficacy data to the EPA. Usage pattern parameters are incorporated into the EPA-registered labels of disinfectant products.

The efficacy of QACs, also known as “quats,” as disinfectants and sanitizers has been studied. Benzalkonium chloride was the first commercially available quaternary compound introduced in 1935. It is widely believed that the mode of action of QACs occurs at the cell wall. By nature, the cell wall of bacteria, fungi, and viruses are negatively charged. The negatively charged cell wall of the organism is attracted to the cationic charge of the QAC molecule. The QAC denatures the protein in the cell wall causing lyses and leakage of intracellular contents.

QACs comprise repeating alkyd groups around a nitrogen atom core. Variations of QACs are generated based on length, designation, and bonding of the repeating alkyd groups. These variations in alkyd functionality provide for various performance characteristics, such as toxicity, biological efficacy, hard water effectiveness, organic soil load tolerance, or detergency.

Organic soil load tolerance is an environmental parameter used to evaluate disinfectants performance on organic matter. Broad-spectrum and hospital disinfectants are tested using a minimum of 5% bovine serum to the AOAC Use-Dilution Test Method for Disinfectants. Organic soil load does not negate precleaning steps for gross filth

Reported by
Annette Baker

Fiberlock Technologies, Inc.,
Andover, Massachusetts

and debris before disinfection. Rather, it is an assurance that any remaining small amounts of organic matter do not deactivate the disinfectant. Dilutions of chlorine bleach (sodium hypochlorite) are easily inactivated by organic matter.

Product disinfectant and sanitizer claims are monitored and regulated by the EPA. Disinfectants must kill 100% of the microorganisms in a specified time.⁽⁴⁾ Sanitizers used on nonfood contact surfaces must reduce the number of microbes by at least 99.9% within a specified time limit.⁽⁵⁾ Products must prove control of a required set of bacteria under specified conditions before they can claim to be disinfectants or sanitizers. Products are tested using AOAC test methods as designated by the EPA.

The EPA guidance states that sanitizers reduce, but not necessarily eliminate, microorganisms from the inanimate environment to safe levels.⁽⁶⁾ The versatility of the product is greatly improved if the product has EPA-registered sanitizer and disinfectant claims. The product can then be applied to porous, semiporous, and hard nonporous surfaces with confidence of the product effectiveness.

Typical water intrusion cleanups require contractors to dry out the affected areas in addition to cleaning. The protocols are adjusted depending on types of surfaces affected, length of time of water intrusion, and contamination found. Semiporous and porous substrates are removed and discarded whenever possible if severely damaged by water.⁽¹⁾

Category 3 water is grossly contaminated and can contain pathogenic, toxigenic, or other harmful agents. Examples of Category 3 water sources include sewage, toilet backflows, and all forms of flooding. Category 3 cleanups use disinfectants as part of the cleaning regimen. Standard Category 3 cleanup projects utilize either pump-up sprayers, or electrical or gas-powered, low-pressure spray equipment to spray disinfectant at low pressure and room temperature conditions.⁽¹⁾

When selecting a disinfectant it is important to consider rigorous environmental application and usage factors that

are present at the jobsite to influence disinfectant activity. Principal factors to consider are: toxicity, high organic soil load, substrate pH, product corrosiveness, hard water tolerance (if concentrate), cleaning ability, flammability, contact time, temperature, and types and density of organisms present. Various principal factors affecting disinfectant activity are summarized in Table I.

METHODS

This section describes the methods used to test the efficacy of QAC for disinfecting Category 3 contamination under challenging application and environmental conditions. First, the beta site selection and conditions are discussed. Then, the disinfectant selection is justified. Finally, the disinfectant application protocol is described.

A beta site was selected that exhibited Category 3 water intrusion resulting from the flood water of Hurricane Katrina. The beta site displayed visible biofilm and exhibited the most severe contamination levels in the facility. The amount and type of contamination as summarized in Table II would indicate usage of a disinfectant not readily affected by heavy biofilm and having a 98% organic soil load efficacy. Further, it was determined that the site would require an aggressive cleaning protocol necessitating deviation from standard disinfectant application process.

Tap water was not available at the beta site, requiring shipment of potable water from a local parish municipality. The estimated water hardness level in New Orleans based on the U.S. Geological Survey is approximately 121–180 ppm.⁽⁷⁾

The beta site exhibited the required variety of substrates of wood, cinder block, concrete, and metal. The site was 2000 ft² (186 m²) of contaminated surface consisting of an L-shaped corridor, including two restrooms conveniently located on the first floor. Accessibility was needed to accommodate pressure washing equipment for testing disinfectant at high temperature

TABLE I. Environmental and Application Considerations for Disinfectants and Soap

Properties	Chemistry				
	Quats	Phenol	Bleach ^A	Alcohol (IPA)	Soap ^B
Affected by pH ⁽¹⁴⁾	Good	Poor	Poor	Good	Fair
Corrosive to equipment	Good	Fair	Poor	Good	Best
Unpleasant odor	Good	Poor	Poor	Poor	Best
Skin irritant at use dilution	Fair	Poor	Poor	Poor	Good
Organic soil tolerance ⁽¹⁴⁾	Fair	Fair	Poor	Poor	Poor
Hard water tolerance ⁽¹⁵⁾	Fair	Fair	Poor	Good	Poor
Cleaning ability ^C	Good	Poor	Fair	Poor	Good
Flammable	Good	Good	Good	Poor	Good
Antimicrobial effectiveness ⁽¹⁶⁾	Best	Best	Fair	Poor	Poor

Note: IPA=isopropyl alcohol.

^AEPA registered 5.25% NaOCl.

^BAntimicrobial dish detergent.

^CNot to include stain removal.

TABLE II. Beta Site Pre-Treatment Sample Analysis

Sample	Final Aerobic Bacteria Culture Screen (CFU/mL)				Final Fungal Culture Screen (CFU/mL)							
	Mixed Bacteria*	Gram-Negative Rods (nonfermentative)	<i>Pseudomonas aeruginosa</i> (presumptive)	Gram-Negative Rods (enteric)	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Aspergillus ochraceus</i>	<i>Aspergillus versicolor</i>	<i>Trichoderma</i> spp.	<i>Rhizopus</i> spp.	<i>Penicillium</i> spp. (morphotype 1)	<i>Fusarium</i> spp.
1	4.0×10^5	2.0×10^5	8.0×10^4	4.0×10^4	none	none	none	1.6×10^4	8.0×10^3	2.0×10^1	none	none
2	6.0×10^5	3.6×10^5	5.0×10^4	1.4×10^4	1.0×10^4	none	2.0×10^3	none	none	2.0×10^1	1.4×10^5	none
3	6.0×10^5	3.2×10^5	7.0×10^4	none	none	none	none	none	8.0×10^3	2.0×10^1	1.2×10^4	2.0×10^1
4	4.0×10^5	2.0×10^5	none	4.0×10^4	none	2.0×10^3	2.0×10^3	none	2.0×10^3	2.0×10^1	9.6×10^4	2.0×10^1

*Mixed bacteria including nonfermentative gram-negative rods.

and pressure. Finally, the facility that housed the beta site was established as a high priority requiring immediate evaluation and restoration.

The condition of the beta site as a result of Katrina flooding was severe. The contaminated water had risen at the beta site to approximately 6 ft (1.83 m) lasting for $2\frac{1}{2}$ weeks. After water levels subsided, the site was left with 3 to 5 ft (0.91 to 1.52 m) of marsh mud. Suspected contaminants included bacteria, viruses, fungi, heavy metals, and inorganic solvents. After removal of the sludge, a greasy biofilm remained covering all substrates in contact with the contaminated water.

Beta site sample methodology used surface swab samples collected at 100 ft (30.48 m) intervals on different substrates at different heights on the walls. Substrates were either semiporous or hard nonporous. Samples were neutralized on collection and sent to a lab for culturing. Four samples of contaminated substrates were cultured and evaluated by an independent, accredited laboratory.

Diagnostics used environmental health screens utilizing cultured samples, particulate analysis, and volatile organic content (VOC) monitoring. Cultures were analyzed for gram-negative and positive bacteria, and parasites. Gram-negative testing was done down to the species level. Various substrates were sampled and evaluated for volatile organic compounds (VOCs) with gas chromatograph/mass spectrometer for hydrogen sulfide contamination. Diagnostics also included an intelligence piece for visual and safety concerns. Substrates were visually inspected for fungal growth.

Forensic and sample methodologies were based on various guidance documents, books, and agencies, such as IICRC S500,⁽¹⁾ *NYC Guidelines*,⁽⁸⁾ EPA's mold remediation guidelines,⁽⁹⁾ and *Bioaerosols*.⁽¹⁰⁾

Given the condition of the beta site, the disinfectant selected would need a high degree of thermal and pressure tolerance, and effectiveness in hard water. The chosen disinfectant would need to address high levels of contamination as noted in Table II.

Several EPA-registered disinfectants with the stated label claims for hard, nonporous surfaces were considered for this application. EPA-registered bleach was not feasible due to higher price per gallon dilutions compared with QAC concentrates, in addition to being corrosive to electrical components. Alcohol was not feasible due to flammability issues. EPA-registered antibacterial (sanitizer) soap and water solutions do not effectively kill all microbes found in Category 3 contaminated residues. Phenol and aldehyde actives have higher levels of toxicity than QACs. It was determined that a QAC based disinfectant/sanitizer would meet stated objectives, while reducing toxicity to the applicator. Disinfectant properties considered in selecting the beta site disinfectant chemistry is summarized in Table I.

The selected QAC consisted of 2.50% dimethyl benzyl ammonium chloride and 2.50% dimethyl ethylbenzyl ammonium chloride mixture⁽¹¹⁾ (Fiberlock Technologies, Inc., Boston, Mass). This QAC was chosen over other QACs due to

packaging in economical concentrate that was extremely versatile as both a sanitizer and disinfectant with efficacy on dilution, with up to 700 ppm hard water and 98% organic soil load tolerance as stated on the EPA-registered label. The EPA-registered label summarized efficacy on more than 130 microorganisms in addition to having efficacy specifically for black water (Category 3) applications. Application temperature of the selected QAC was amenable to both cold and hot pressure washers and could withstand higher application pressures.

The approach to the disinfectant application at the beta site was to "shock" the building using hot, high-pressure washing of the cinder block walls, concrete floors, wood work, metal doors, and all cleanable contaminated surfaces. Noncleanable surfaces were discarded. Pressure washing crews worked 10 to 15 hours per day. After initial pressure washing was completed for gross filth and debris, the substrates were allowed to air dry and samples taken to ascertain effectiveness of the "shocking" wash. The results yielded a reduction of contaminants by 50%.

Because tap water was not available, potable water was shipped to the beta site in tankers. Potable water was then transferred to a 500-gallon (1893-L) reservoir for mixing with disinfectant concentrate. Eight gallons of disinfectant was added to the reservoir first with top filling of water to disperse disinfectant concentrate. The 500-gallon reservoir and pressure washer was trailer mounted for portability (model 8307; Alkota Cleaning Systems, Inc., Alcester, S.D.). The skid-mounted pressure washer featured a diesel drive Lombardini 23.4 HP engine with a 115 volt generator.

The disinfectant mixture was transferred via 100 ft (30.5 m) of high-pressure hose to a high-pressure wand equipped with a 60° spray pattern. The outlet pressure at the nozzle was set for 3000 psi (20,684 kPa) at 8 gallons/min (30.28 L/min) with operating temperature of 140°F (60°C). Disinfectant was evenly distributed using sweeping motions from side to side approximately 16 inch (41 cm) from the substrate. During the final cleaning, the disinfectant was provided a 10-min contact time before the substrate was allowed to air dry. Enzyme testing methodology was used to test for fungi and to determine whether additional passes were needed.

Enzyme testing methodology utilized the MycoMeter test system (MycoTec ApS, Copenhagen). Surface samples were taken by swabbing then processed for analysis by extraction and transferred to cuvette for fluorometer reading. The amount of enzyme activity present is directly proportional to biomass density, thereby quantifying amount of fungi. When the MycoMeter yielded fluorescence counts of 25 (FC) or higher, additional cleaning passes were conducted.

Three phases of cleaning were utilized to gain acceptable results at the beta site:

- Phase I—Removal of gross filth and debris
- Phase II—Disinfectant, hot, high-pressure wash "shocking"
- Phase III—Follow-up disinfectant hot, high-pressure wash and/or manual wipe down.

TABLE III. Beta Site Bacterial Sample Analysis Before and After Treatment

Final Aerobic Bacteria Culture Screen, cfu/ml									
Beta Site Pre-Treatment Sample Analysis (Before)					Beta Site Post-Treatment Sample Analysis (After)				
Sample	Mixed Bacteria*	Gram-Negative Rods	<i>Pseudomonas aeruginosa</i> (presumptive)	Gram-Negative Rods (enteric)	Sample	Mixed Bacteria*	Gram-Negative Rods	<i>Pseudomonas aeruginosa</i> (presumptive)	Gram-Negative Rods (enteric)
1	4.0×10^5	2.0×10^5	8.0×10^4	4.0×10^4	1	4.0×10^1	none	none	none
2	6.0×10^5	3.6×10^5	5.0×10^4	1.4×10^4	2	4.0×10^1	none	none	none
3	6.0×10^5	3.2×10^5	7.0×10^4	none	3	$>6.0 \times 10^5$	none	1.6×10^4	1.2×10^4
4	4.0×10^5	2.0×10^5	none	4.0×10^4	4	3.6×10^5	2.4×10^5	none	1.0×10^4

*Mixed bacteria including nonfermentative gram-negative rods.

RESULTS

Direct exam results of sample areas at the beta site prior to cleaning revealed fungal content having moderate and rare conidia/spores with many bacterial rods/cocci and few parasitic ciliates and flagellates. Bacterial and fungal analysis of the beta site prior to cleaning showed levels of contamination as summarized in Table II. Table II indicates gram-negative contamination ranging from 1.4×10^4 to 6.0×10^5 colony forming units/mL. Fungal counts revealed significant amounts of *Aspergillus*, *Trichoderma*, *Rhizopus*, *Penicillium*, and *Fusarium* species.

Beta site post-treatment enzyme testing results concluded the substrates were being cleaned properly with careful monitoring by staff. Micro assessments were used in addition to enzyme testing after final wash. Micro assessment included white glove test and swabbing. If either glove or swab were found to have debris fields, a disinfectant wipe down would then be incorporated for the area in question.

Post-cleanup samples were taken using the sample collection strategy and methodology utilized during preliminary contamination testing. Fungal analysis included quantitation of bacteria and fungi, along with identification of up to three significant pure or predominant fungi. Additional molds were mentioned or identified to clarify quantitation. Bacteria was quantitated but not identified. The enteric bacteria screen included determination of the presence or absence of enteric bacterial pathogens and quantitation.

Beta site post-treatment direct exam results revealed fungal content having few conidia/spores and bacterial rods/cocci and no parasites seen. Bacterial and fungal analysis of beta site indicated significantly reduced contamination levels as summarized in Tables III and IV.

Table III reviews the elimination of nonfermentative and enteric bacteria in Samples 1 and 2. *Pseudomonas aeruginosa* dropped from 8.0×10^4 to none in Sample 1 and 5.0×10^4 to none in Sample 2. Sample 3 showed an increase in enteric bacteria from none to 1.2×10^4 after treatment. Sample 4 showed a slight increase in nonfermentative gram-negative rods after treatment, but enteric bacteria was reduced. Overall, before and after treatment analysis shows a reduction or elimination of gram-negative bacteria.

Table IV summarizes effective fungal reduction before and after treatment. Sample 1 indicates the elimination of *Aspergillus versicolor*, *Trichoderma* spp., and *Rhizopus* spp. Samples 2 and 4 shows fungal reduction or elimination. Sample 3 indicates elimination of *Trichoderma* spp., *Rhizopus* spp., *Penicillium* spp., and *Fusarium* spp. However, *A.versicolor* was noted in Sample 3 at 4.0×10^1 after treatment but was not present in pre-treatment analysis. Post-treatment results indicate the overall reduction or elimination of fungal conidia/spores after application of the disinfectant protocol.

DISCUSSION

The cleaning protocol addressed disinfection effectiveness, economics, and ease of application. The selected QAC was economically packaged as a concentrate yielding 64 gallons of usable product on dilution. The QAC was easy to apply, having efficacy for hot or cold and low- or high-pressure spray equipment.

The QAC active disinfectant was tested using a reproducible cleaning protocol, and effectiveness was confirmed by beta site sample analysis by an independent accredited laboratory. Application of EPA-registered quaternary ammonium active having 98% organic soil load and hard water efficacy was challenged with elevated application conditions of high spray application pressure and temperature.

The results from post-treatment sample analysis show the QAC active disinfectant was effective in disinfecting Category 3 contamination at the higher than standard application temperature of 140°F (60°C) and 3000 psi (20,684 kPa) outlet spray pressure. The QAC evaluated showed efficacy under dynamic field trial conditions of high organic soil load caused by Category 3 water intrusion and elevated water hardness when diluted with potable water.

After beta site cleaning methodology was validated, the remainder of the building that housed the beta site was cleaned using the disinfectant protocol. Only the first floor of the building utilized the disinfectant cleaning protocol, as flood waters did not reach the second floor. The first floor square footage treated with the disinfectant protocol is estimated at 15,000 ft² (1394 m²), not including beta site. The affected areas deemed unsalvageable were removed. The total estimated area

TABLE IV. Beta Site Fungal Sample Analysis Before and After Treatment

Final Fungal Culture Screen (CFU/mL)												
Beta Site Pre-Treatment Sample Analysis (Before) Screen (CFU/mL)						Beta Site Post-Treatment Sample Analysis (After)						
Sample	<i>Aspergillus niger</i>			<i>Aspergillus ochraceus</i>			<i>Aspergillus versicolor</i>			<i>Trichoderma Rhizopus</i>		
	<i>flavus</i>	<i>Aspergillus niger</i>	<i>Aspergillus ochraceus</i>	<i>Aspergillus niger</i>	<i>Aspergillus ochraceus</i>	<i>Aspergillus versicolor</i>	<i>Trichoderma</i>	<i>Rhizopus</i>	<i>Penicillium</i> spp. (morphotype 1)	<i>Fusarium</i> spp.	<i>Penicillium</i> spp. (morphotype 1)	<i>Fusarium</i> spp.
1	None	None	1.6×10^4	None	8.0×10^3	2.0×10^1	None	2.0×10^1	None	None	None	None
2	1.0×10^4	None	2.0×10^3	None	None	2.0×10^1	1.4×10^5	2.0×10^1	None	None	None	1.0×10^2
3	None	None	None	None	8.0×10^3	2.0×10^1	1.2×10^4	2.0×10^1	2.0×10^1	None	None	None
4	None	2.0×10^3	2.0×10^3	None	2.0×10^3	2.0×10^1	9.6×10^4	2.0×10^1	2.0×10^1	None	None	4.0×10^1

TABLE V. High School Post-Treatment Sample Analysis

Final Aerobic Bacteria Culture Screen (CFU/mL)		Final Fungal Culture Screen (CFU/mL)																							
Sample	Mixed Bacteria* (nonfermentative)	Gram-Negative Rods		Gram-Negative Rods (enteric)	Sample	Acremonium spp.		Aspergillus <i>flavus</i>		Aspergillus <i>niger</i>		Aspergillus <i>ochraceous</i>		Aspergillus <i>versicolor</i>		Trichoderma spp.		Rhizopus spp.		Penicillium spp. (morphotype 1)		Fusarium spp.			
		<i>Pseudomonas aeruginosa</i> (presumptive)	<i>aeruginosa</i>			2.0 × 10 ¹	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
1	8.0 × 10 ¹	None	None	None	1	2.0 × 10 ¹	None	None	None	None	None	None	None	4.0 × 10 ¹	None	None	None	None	None	None	None	None	None	None	None
2	None	None	None	None	2	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
3	None	None	None	None	3	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
4	None	None	None	None	4	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None
5	None	None	None	None	5	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	2.0 × 10 ¹

*Mixed bacteria including nonfermentative gram-negative rods.

with contamination on the first floor was 30,000 ft² (2787 m²); estimation includes beta site. The second floor of the facility was checked using environmental health screens. As a safety measure, a disinfectant wipedown was used on all second floor surfaces.

After post-treatment, direct exam results taken from samples on the first floor showed minimal visual fungal elements and no parasitic or bacterial content. Independent lab analysis of the ground floor facilities summarized in Table V shows limited contamination after treatment. Sample analysis indicates no nonfermentative and enteric gram-negative bacteria found in all five samples. Fungal results showed *Acremonium* spp. at 2.0×10^1 and *A. versicolor* at 4.0×10^1 in Sample 1. Sample 5 exhibited 2.0×10^1 of *Penicillium* spp. Samples 2, 3, and 4 exhibited no fungal contamination found.

CONCLUSION

The beta site cleaning methodology was tested and disinfectant efficacy was confirmed. Application of the disinfectant protocol was utilized at the remainder of the high school facility on the ground floor confirming beta site protocol effectiveness and results. The effective remediation of Category 3 water contamination using the stated disinfectant cleaning protocol under challenging application and environmental conditions was confirmed.

A scope of work was developed for the remediation of 21 schools utilizing the cleaning and disinfectant protocol of the beta site.

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