ENVIRONMENTAL CONTROL IN BIOLOGICAL PRODUCTION ROOMS UTILIZING VAPORIZED HYDROGEN PEROXIDE (VHP®) AND ULTRA-VIOLET BIODECONTAMINATION APPLICATIONS

INTRODUCTION

Figure 1. STERIS Corporation VHP 1000ED Generator.

Cleaning and biodecontamination of production aseptic processing rooms has traditionally been performed using chemical disinfectants such as sodium hypochlorite and commercial detergents. In general, liquid disinfectants are not a completely thorough process and recontamination of microbials such as yeasts and molds are a continuing problem. Gaseous processes such as formaldehyde and chlorine dioxide prove to be labor intensive and produce significant health and safety concerns. The application of Vaporized Hydrogen Peroxide (VHP) and Ultra-Violet (UV) processes has gained wide acceptance for cleanroom applications. Common outbreaks of microbial contaminants can be decontaminated* with the application of Vaporized Hydrogen Peroxide. In this case study, 2,000 cubic foot production rooms that are used for *Bacillus* spore processing in the production of Commercial Biological Indicators (BIs) are decontaminated using UV light sources and a VHP 1000ED Generator with specifically developed cycles for this application. The study objective is to determine and compare the efficacy of VHP and Ultra-Violet room biodecontamination applications effectiveness.

METHODS

Room Specifications

The room volume for this testing study was 2,000 cubic feet. Two oscillating fans were dispersed randomly to aid in vapor distribution during test cycles. Vent airflow was turned off during the test cycles to maintain vapor concentrations throughout the test area. Vents were sealed to prevent gas going into the ventilation system. Electrical equipment normally found in the rooms, such as centrifuges, vortex mixers, and laminar flow hoods, were left in the room during testing.

Bio-Burden Samples

Bacillus stearothermophilus (ATCC#7953) and *Bacillus subtilis variant niger* (NRRL#B4418) spore coupons were prepared by STERIS Corporation, Mentor, Ohio. *B. stearothermophilus* spores were selected as the primary test organism because of their known acceptance as the most resistant organism to the Vaporized Hydrogen Peroxide process[1]. Organism samples were randomly placed throughout the test rooms and locations recorded. Population enumeration was performed on samples through serial dilution, inoculating on Tryptic Soy Agar (TSA), and counting of Colony Forming Units (CFUs). Mean counts of 2.2×10^6 for *B. stearothermophilus* spores and 2.1×10^6 for *B. subtilis* were established prior to sterilant exposure.

Ultra-Violet Exposure

The 2,000 cubic foot rooms used for testing were installed with fixed wall mounted UV lamps installed in the middle of the longitudinal walls approximately 6 inches from the ceiling surface. Each wall unit consists of four UV bulbs with 36 watt specification. Distances from the biological sample sites were measured and recorded. UV exposure time was for 8 hours.

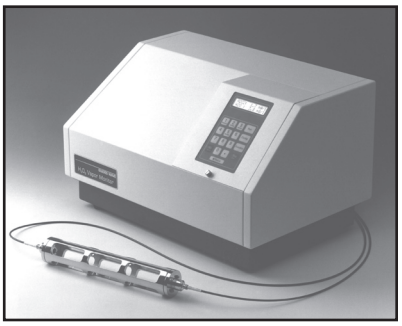

Figure 2. UOP Guided Hydrogen Peroxide Sensor

METHODS

VHP Sterilant Exposure

The 2,000 cubic foot rooms used for testing were installed with two wall portals outside the room for connection to a VHP 1000ED Generator (Figure 2), one used for inlet of VHP gas, the other used for airflow return to the generator. A third portal was installed for sensor probe lines to a UOP Guided Wave Hydrogen Peroxide Sensor (Figure 3) to monitor concentration levels during the biodecontamination cycle. Prior to biological sample exposure, a mean room concentration of hydrogen peroxide was established at 1mg/L +/- .2 mg/L. A dose response curve was established inside a 22 ft^3 flexible isolator for concentration over time exposure for *B. stearothermophilus* spores to aid in determining cycle time (Figure 1). Cycle parameters were developed and optimized for the test room. The VHP 1000ED Generator was programmed to the following parameters for the 2,000 cubic foot production room biodecontamination cycle:

Dehumidification Phase:	20 SCFM 45 Minutes
Conditioning Phase:	20 SCFM 5 Minutes 12 g/min Injection Rate
Biodecontamination Phase:	20 SCFM 60 Minutes 10 g/min Injection Rate
Aeration Phase:	20 SCFM 60 Minutes
Total Time:	2 Hours 50 Minutes

VHP Vapor Distribution and Temperature Mapping

Temperature throughout the test room areas was monitored and recorded with remote thermometer probes. Oscillating fans were placed in the test area to aid in vapor dispersion. Vapor distribution through test areas was qualitatively monitored using STERIS VHP Chemical Indicators. Vapor was quantitatively monitored using a UOP guided wave H_2O_2 monitor.

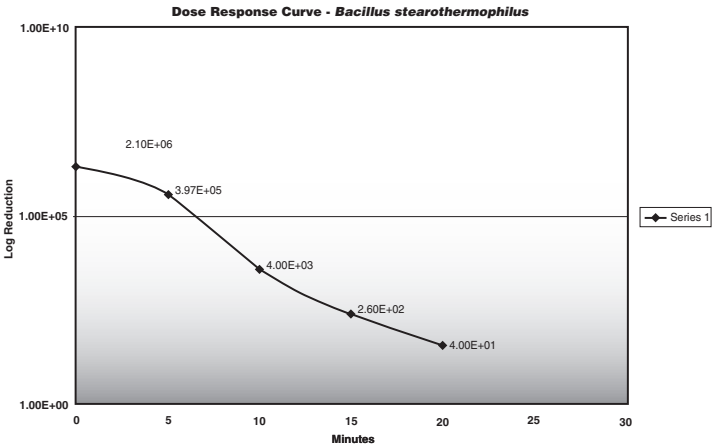

Figure 3. Dose Response Curve for 1.0 mg/L Exposure for Biological Indicators

Biological Sample Processing

After VHP and UV exposure, inoculated carriers of both strains were aseptically harvested and placed in 10mL of Tryptic Soy Broth (TSB). A 1 mL sample was removed, serial diluted, and plated on TSA for possible population enumeration. The remaining solution was incubated (37°C for *B. subtilis* and 55°C for *B. stearothermophilus*) for seven days. Growth checks were performed daily.

RESULTS

Vapor Distribution

Temperature ranges inside the test rooms were 20 - 25°C during biodecontamination cycles. Vapor distribution throughout the test rooms was verified by chemical indicator color change. All chemical indicators within the testing rooms showed a positive change indicating the presence of hydrogen peroxide. UOP sensor readings for H_2O_2 reached a maximum level of 1.1 mg/L during the biodecontamination phase of the cycle.

Exposed Biological Results

B. stearothermophilus and *B. subtilus var. niger* inoculated coupons (at >10^6 spores/coupon) were placed at various locations in the 2,000 cubic foot room and exposed to either VHP or UV biodecontamination cycles. Results are shown in Tables 1 to 4.

Test Organism/Sample No.	Log Reduction	Distance from UV Lamp
B. stearothermophilus-1	10^3	6 ft.
B. stearothermophilus-2	10^3	6 ft.
B. stearothermophilus-3	10^2	8 ft.
B. stearothermophilus-4	10^2	8 ft.
B. stearothermophilus-5	10^1	10 ft.
B. stearothermophilus-6	10^2	10 ft.
B. stearothermophilus-7	0	12 ft.
B. stearothermophilus-8	10^1	12 ft.
B. stearothermophilus-9	10^2	10 ft.
B. stearothermophilus-10	0	10 ft.

Table 1. Biodecontamination Log Reductions of *B. stearothermophilus* utilizing UV in 2,000 cubic foot test room.

Test Organism/Sample No.	Log Reduction	Distance from UV Lamp
B. subtilis-1	10^3	6 ft.
B. subtilis-2	10^3	6 ft.
B. subtilis-3	10^3	8 ft.
B. subtilis-4	10^2	8 ft.
B. subtilis-5	10^1	10 ft.
B. subtilis-6	10^2	10 ft.
B. subtilis-7	0	12 ft.
B. subtilis-8	0	12 ft.
B. subtilis-9	10^2	10 ft.
B. subtilis-10	0	10 ft.

Table 2. Biodecontamination Log Reductions of *B. subtilis* utilizing UV in 2,000 cubic foot test room.

Test Organism/Sample No.	Reduction
B. stearothermophilus-1	10^6
B. stearothermophilus-2	10^6
B. stearothermophilus-3	10^6
B. stearothermophilus-4	10^6
B. stearothermophilus-5	10^6
B. stearothermophilus-6	10^6
B. stearothermophilus-7	10^6
B. stearothermophilus-8	10^6
B. stearothermophilus-9	10^6
B. stearothermophilus-10	10^6

Table 3. Biodecontamination Log Reductions of *B. stearothermophilus* utilizing VHP in 2,000 cubic foot test room.

Test Organism/Sample No.	Reduction
B. subtilis-1	10^6
B. subtilis-2	10^6
B. subtilis-3	10^6
B. subtilis-4	10^6
B. subtilis-5	10^6
B. subtilis-6	10^6
B. subtilis-7	10^6
B. subtilis-8	10^6
B. subtilis-9	10^6
B. subtilis-10	10^6

Table 4. Biodecontamination Log Reductions *B. subtilis* utilizing VHP in 2,000 cubic foot test room.

DISCUSSION

Rooms were entered after proper aeration of H_2O_2 was completed for VHP applications. Biological samples were aseptically processed. After samples were collected, rooms were inspected and no visible or textural change in surfaces was detected after VHP exposure. Some plastic discoloration was noted on some areas after exposure to UV. All electrical equipment was functioning properly and no damage detected for both decontamination applications.

The Ultra-Violet application did not successfully reduce the test bio-burden. It was most effective at the shortest distance sites. At greater distance sites, UV was minimally effective or ineffective. At higher concentration contamination, UV would not be the best means of total decontamination but could be used in conjunction with a more thorough process application. The variability of bio-burden reduction may prove difficult in validating this type of process. The exposure time of 8 hours should also be considered as a constraint in this type of application.

The VHP application, which used the same sample site areas, completely reduced all test samples of both spore strains. Because the process allows for equal distribution of vapor throughout the total volume of the room, the assurance level would be quite high in this type of decontamination application. The achievement of complete reduction of the bio-burden at various locations could be easily validated as a process control. The exposure time, which should be a consideration in applying any decontamination process, was 60% less than the UV application.

A decontamination application should not be intended as a substitute for routine cleaning processes. Preventive maintenance cleaning of work areas and surfaces should be completed prior to any decontamination application. Cleaning methods should also be verified and monitored.

CONCLUSION

The antimicrobial efficacy of the VHP 1000ED room biodecontamination cycle was sufficient to completely reduce test bioburdens of six logs of *B. subtilis variant niger* and *B. stearothermophilus* spores. In critical environments, a Vaporized Hydrogen Peroxide application can prove to be both an effective and efficient method of environmental control.

Advantages to VHP Room biodecontamination:

1) It is compatible with many types of material, including sensitive electrical equipment.

2) Rapid cycle time and turnaround time.

3) The process is adaptable and easily controlled.

4) The process is easily validated to meet user need.

5) The sterilant decomposes to environmentally friendly products of water and oxygen.

6) Personnel safety and health issues are minimized because of containment.

7) May be used in conjunction with various cleaning agents.

REFERENCE

1. Kokubo M, Inoue T, and Akers J, 1998, “Resistance of Common Environmental Spores of the Genus Bacillus to Hydrogen Peroxide Vapor,” PDA, J. Pharm. Sci. Technol., 52: 228-231.

*When using VHP equipment with Vaprox Hydrogen Peroxide Sterilant in the United States, the team biodecontamination referred to in this document is defined as sterilization of exposed porous and non-porous surfaces in a pre-cleaned, dry, sealed enclosure. Any reference to biodecontamination as it relates to the use of this equipment in the United States does not impart additional claims of effectiveness beyond that approved in the EPA-registered labeling of Vaprox Hydrogen Peroxide Sterilant.

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