VAPORIZED HYDROGEN PEROXIDE BASED BIODECONTAMINATION OF A HIGH-CONTAINMENT LABORATORY UNDER SLIGHT NEGATIVE PRESSURE

Office of Biosafety & Environment
Jay Krishnan, Greg Fey, Stefan Wagener

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INTRODUCTION

The Canadian Science Centre for Human and Animal Health (CSCHAH) houses sixteen animal cubicles, five containment level 3 (CL3) suites and seven CL4 suites each containing individual laboratories. Since its opening in 1997, formaldehyde gas has been used for decontaminating the containment laboratories and biosafety cabinets. This process has often been slow, disruptive and difficult to standardize (1;2). Additionally, formaldehyde gas polymerizes to paraformaldehyde and settles on the surfaces, warranting thorough post-decontamination clean up following neutralization. Some porous materials such as wood, paper and clothing absorb, retain and release formaldehyde gas over time (3). In addition to being a health hazard (4;5), a mixture of formaldehyde gas or paraformaldehyde dust in air has the potential to explode (6;7). Therefore, we have been exploring alternate safer and automated technologies for area decontamination.

Vaporized hydrogen peroxide (VHP) is a relatively new type of biodecontamination technology (8) was developed in the 80's and commercialized in the early 90's. VHP is a powerful oxidizer and it kills a variety of microbes and bacterial spores at low concentrations. The process is rapid, mobile and effective at low temperatures, and has been widely used in the pharmaceutical industries. Unlike formaldehyde, VHP produces non-toxic by-products (water and oxygen), therefore is ecologically safe and requires no post-process neutralization and cleaning. However, a VHP concentration of over 75ppm is considered as an immediate risk to human health and the accepted personal exposure level is under 1ppm (9;10).

Here we report three successful biodecontamination processes of a CL3 laboratory (lab) using vaporized hydrogen peroxide. The lab was kept at a slight negative pressure to prevent hazardous levels of VHP leaking

MATERIALS & METHODS

THE LABORATORY

built as a CL3 lab and has a volume of 2893 cubic feet and contains biosafety cabinets (Class II Type A2 & Class III), incubators, refrigerator, freezer, centrifuge, telephone, fax machine, computer, security camera, microscope and other routine lab equipment (all the equipment and electronics remained functional after the biodecontamination processes). For thorough circulation of VHP within the lab-space, four oscillating fans were placed inside (fig. 1). The position and direction of oscillation were determined by an air-current-smoke test. Additionally, the class II BSC was left running during the biodecontamination process to further enhance VHP distribution. The floor-drain, sink-drain and the door to the dirty change room were shut and sealed with poly film & vapor barrier tape before starting the biodecontamination program cycle.

THE VHP GENERATOR

STERIS® VHP 1000ED (fig.2) was placed in the mechanical space above the lab (penthouse). VHP was piped in and the return air piped out of the lab using two 1½ inch stainless steel pipe penetrations in the concrete slab (fig.1).

THE BIODECONTAMINATION PROGRAM CYCLE

Dehumidification to 30% relative humidity (30 minutes), *Conditioning* injection rate 11gm H_2O_2/min (20 minutes), *Decontamination* injection rate 8gm H_2O_2 /min (90 minutes), *Aeration* 60 minutes and the *Flow rate* was set at 20cfm (11)

THE PROCESS CONTROL & MONITORING

A Dräger Polytron 2 transmitter fitted with a high-concentration H_2O_2 electrochemical sensor (fig.3) was set up in the laboratory to monitor real-time VHP concentration (fig.4) via the laboratory security camera and was recorded on to our in-house digital video recording system.

The lab pressure during the process was monitored and logged (fig.5) using an Ashcroft ATE-100 digital manometer.

Steris® VHP Chemical indicators (CI) were placed at different locations (N=45) in the lab (fig.6) to visualize the extent of VHP distribution.

THE SAFETY ASSURANCE

To prevent hazardous VHP from leaking out, the lab pressure was set at approximately minus 50 pascals throughout the biodecontamination process (fig. 5). This was accomplished by slightly opening a manual bioseal damper.

At the end of the program cycle, the poly seal on the grill of the door leading to the dirty change room was broken and the room ventilation turned on to evacuate the remaining VHP overnight.
The following morning, air was sampled in the neighboring labs, rooms and the penthouse mechanical space for the presence of VHP using Dräger gas detection tubes for H_2O_2 (fig. 7).

The lab was entered wearing Self Contained Breathing apparatus to measure the residual VHP using Dräger H_2O_2 tubes. The results showed less than 0.3ppm, well below the personal exposure level and therefore, normal access to the lab was permitted for retrieving the biological & chemical indicators.

THE STERILITY ASSURANCE

To validate the process's extent and efficiency of microbial sterilization within the lab-space, biological indicator (BI) pouches (fig.8) containing >10^6 spores of *Geobacillus stearothermophilus* dried on stainless steel metal discs (Apex Laboratories) were placed at different locations (N=45) within the laboratory. Upon completion of the biodecontamination program, the pouches were retrieved, opened, the discs were inoculated on Tryptic Soy Broth tubes and incubated at 56ºC. An untreated BI was also included as a positive growth control. The cultures were observed for bacterial growth up to 7 days. All cultures remained negative for growth except the positive control, which became positive after overnight incubation.

RESULTS

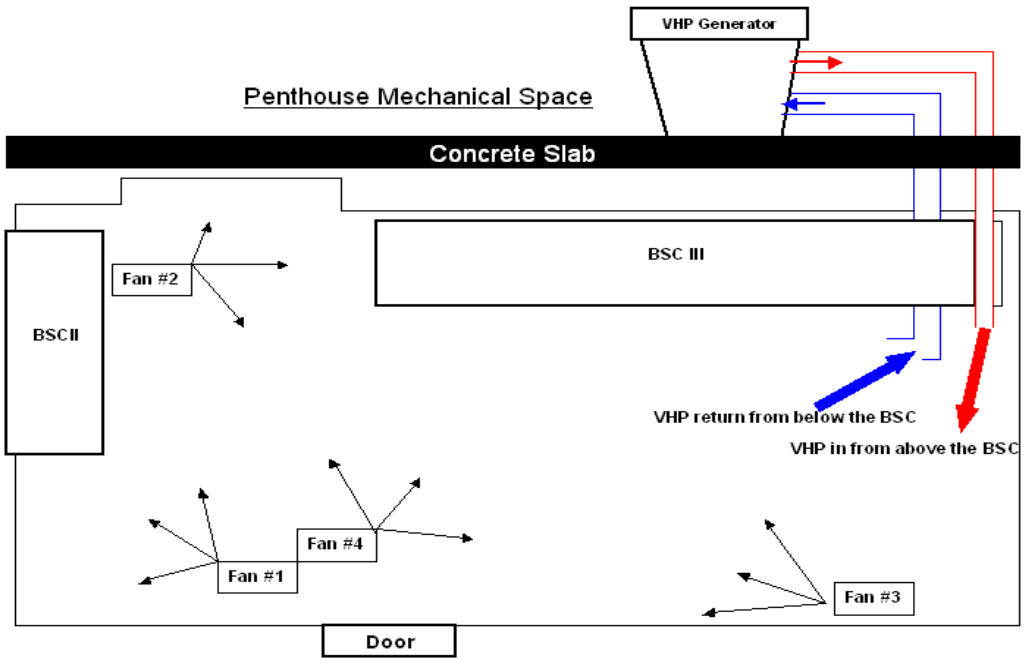

Fig. 1: Laboratory setup for the VHP biodecontamination. Arrows indicate the fans' oscillation directions

Fig. 2: Front view of the VHP generator (STERIS® VHP 1000ED) located on the penthouse mechanical space. VHP was piped into the lab for the biodecontamination process

Fig. 3: Dräger Polytron 2 transmitter and H_2O_2 electrochemical sensors. A high concentration sensor (0-7000ppm) connected to the transmitter was set up in the lab to monitor real-time VHP concentration

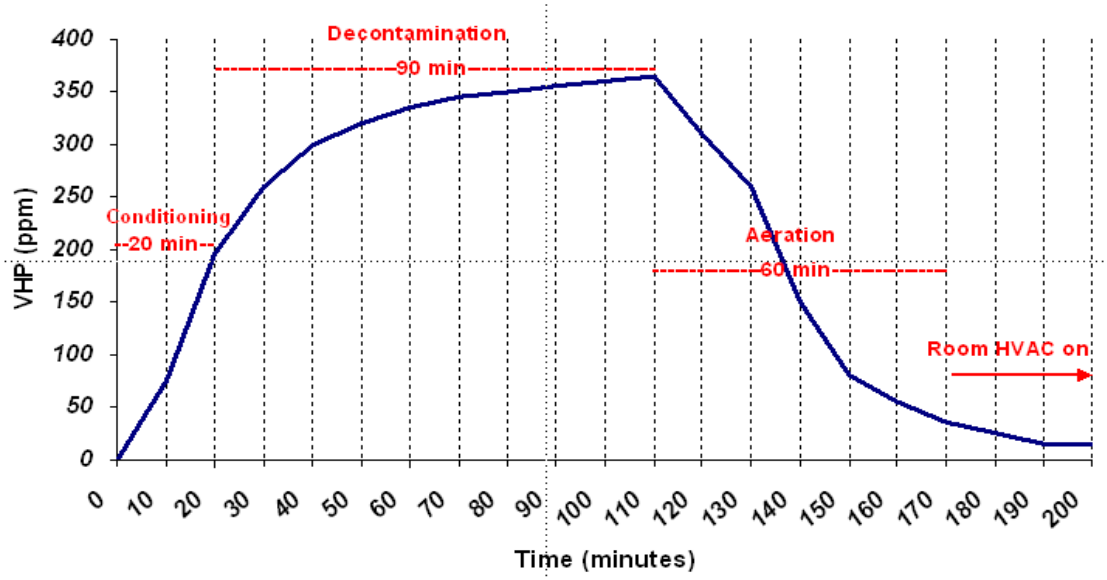

Fig. 4: Real-time VHP concentration recorded in the lab during the three phases of the biodecontamination program cycle. VHP concentration was kept on rising until the end of *decontamination* phase and the peek concentration reached was only 365ppm.

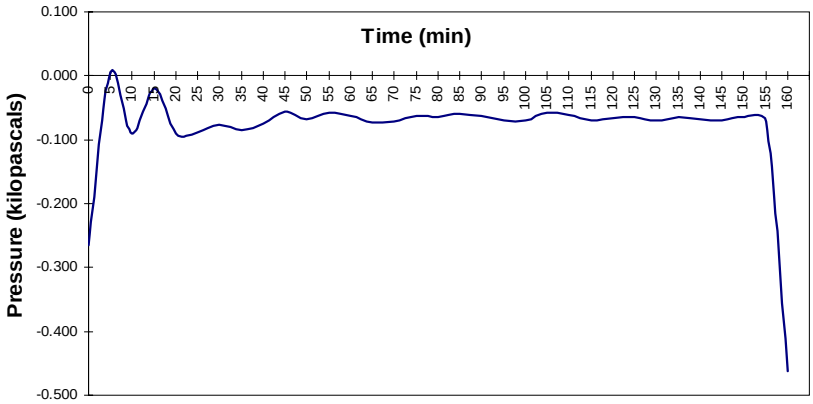

Fig. 5: The laboratory was maintained at a slight negative pressure (minus 50 pascals) to prevent VHP from leaking out. This was achieved by evacuating air from the lab, consequently resulting in a lower concentration of VHP in the lab.

Fig.6: Chemical Indicator: Upon VHP exposure the blue colored indicator becomes beige. All 45 CIs changed color indicating that the VHP reached virtually everywhere in the lab.

Fig.7: Dräger gas detection tubes for H_2O_2: Capable of determining VHP concentration between 0.1-3 ppm (semi quantitative). White crystals in the tube become brownish upon reaction with H_2O_2. After overnight ventilation, the lab read 0.3ppm residual VHP concentration, below the personal exposure level. Air samples in the surrounding areas of the lab tested negative for VHP during & after the biodecontamination process indicating the absence of VHP leak out of the lab.

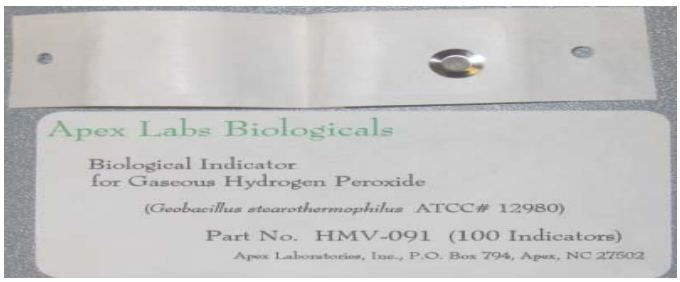

Fig.8: Biological Indicator: Metal disc containing dried *G. stearothermophilus* spores ($2.3X10^6$/disc) in VHP permeable Tyvek pouch. All 45 indicators failed to grow upon incubation up to 7 days at 56ºC, indicating the thoroughness and validity of laboratory sterilization by VHP

CONCLUSIONS

1) VHP biodecontamination of a lab/room under slight negative pressure can be performed safely.

2) Even though we could achieve only a maximum concentration of 365 ppm of VHP in the lab, the process resulted in an over six-log-kill of dried *G. stearothermophilus* spores.

3) VHP biodecontamination was compatible with a variety of routine laboratory equipment and electronics.

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Acknowledgements

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