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Cycle Parameters For Decontaminating A Biological Safety Cabinet Using H_2O_2 Vapor

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ABSTRACT

Several studies have shown that hydrogen peroxide vapor (H_2O_2) can be useful in decontaminating HEPA filters, isolation chambers and centrifuge enclosures.[1-3] However, before hydrogen peroxide can be used reliably in a biological safety cabinet (BSC), it is essential to establish the cycle parameters which allow full decontamination, and which minimize overall cycle time. This paper describes research which established the appropriate physical modifications and decontamination cycle parameters for the Baker Model SG-600, which is a Class II, Type A/B3 biological safety cabinet.

DECONTAMINATION USING H_2O_2 VAPOR

Unlike other common sterilants, hydrogen peroxide is non-carcinogenic and non-mutagenic. The vapor breaks down into oxygen and water, which are both environmentally benign. Current theories hold that the oxygen radical released as the vapor decomposes is responsible for the highly lethal effect of the vapor on microorganisms. The D-values for H_2O_2 (the time required to kill a specified percent of the population) suggest that the vapor can be effective in less time than either formaldehyde or ethylene oxide. Finally, hydrogen peroxide has a long-established role as a liquid disinfectant for surfaces in medical facilities, so many aspects of its behavior are well understood.

In vapor form, however, H_2O_2 behaves differently than the liquid in two useful ways. First, because the molecule is less stable in the vapor form, it decomposes more rapidly than the liquid, releasing more oxygen radicals in a given time. This makes the vapor more effective than the liquid in killing microorganisms. Also, as a gaseous vapor, H_2O_2 can sterilize not just surfaces, but particles suspended in the air as well.

A less desirable aspect of H_2O_2 behavior is the ease with which some materials absorb the vapor. Cellulosic materials such as particle board absorb the vapor during sterilization, then release it slowly, which lengthens the time needed to reduce the concentration of H_2O_2 in a cabinet to the required 1 PPM threshold limit value (TLV). In addition, the same vigorous chemical activity which makes H_2O_2 a useful sterilant can accelerate decomposition of certain materials commonly used in BSC's.

Consequently, before using H_2O_2 to decontaminate a biological safety cabinet, it is important to define the behavior of the vapor for the specific configuration and material composition of each cabinet.

RESEARCH PLAN & PROCEDURES

First, the cabinet materials were tested to determine whether they absorb H_2O_2 vapor and whether repeated exposure causes any reduction in BSC integrity. Typical samples of the materials were sent to AMSCO, where they were exposed to several hundred decontamination cycles.

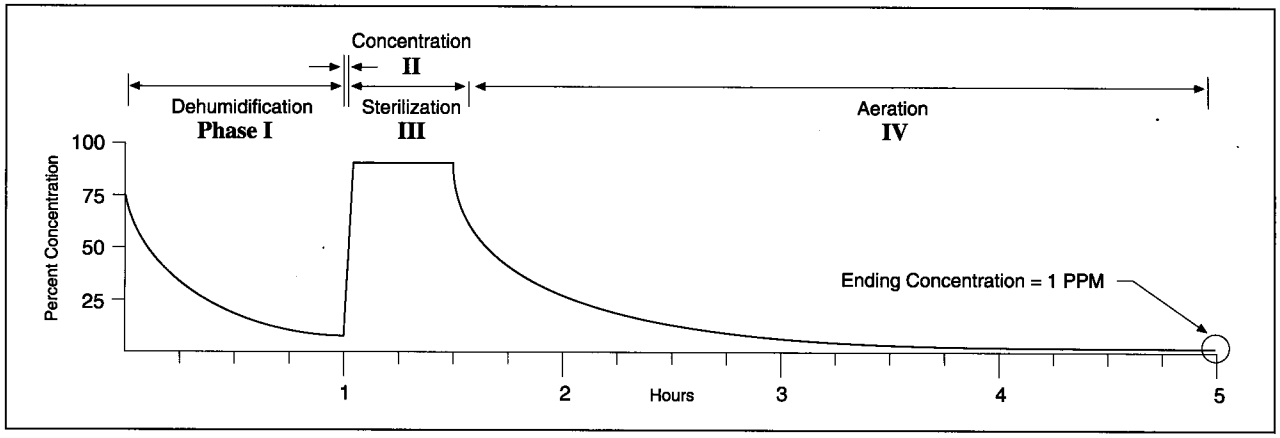

Figure 1. Phases of a typical decontamination cycle which uses hydrogen peroxide vapor.

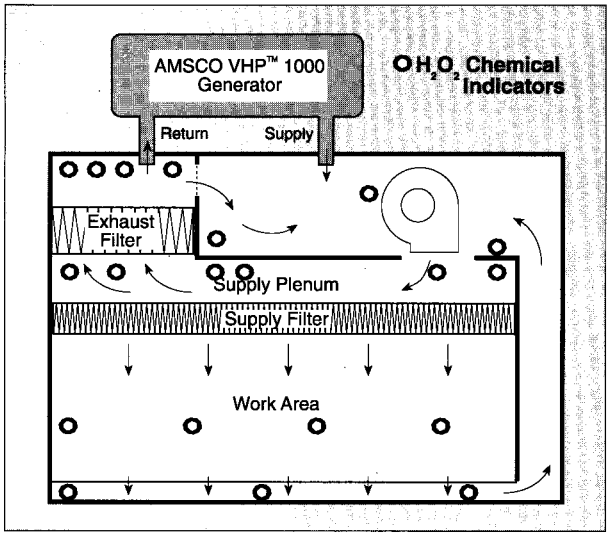

Figure 2. BSC configuration with the locations of chemical indicating strips.

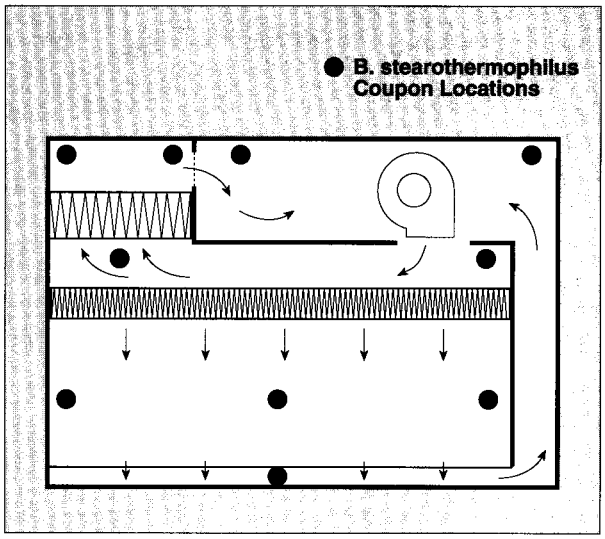

Figure 3. Locations of B. stearothermophilus coupons

Upon their return to Baker, physical and visual observations were made of the color, as well as the structural and operational aspects of the materials. Then these observations were compared to materials in similar cabinets that had not been exposed to H_2O_2.

Next, we investigated the distribution of vapor throughout the cabinet. Because H_2O_2 vapor decomposes within minutes or seconds, it is important to ensure that all corners of the cabinet are accessible to the gas. Otherwise it may take too long for air in all parts of the cabinet to reach H_2O_2 concentrations which are high enough to be effective. Vapor distribution was quantified by placing 20 chemical indicator strips in the locations described on figure 2. Where cabinet components obscured visual inspection of these strips, clear acrylic panels were substituted for metal panels.

After H_2O_2 vapor was injected into the cabinet, the observer recorded the percent of color change on each indicating strip every five minutes. Areas slow to reach effective exposure levels were redesigned and modified so that vapor concentrations could build quickly, shortening the time needed to achieve full exposure. Several alternative designs were evaluated, including: relocating vapor input and output ports, adding more supply and return ports and developing alternate air flow pathways through the cabinet. After flow patterns were optimized, the cycle parameters could each be examined to reduce overall time to a minimum while maintaining decontamination effectiveness.

H_2O_2 decontamination requires four steps; drying the cabinet, concentrating the vapor, fully exposing all surfaces to sterilize the cabinet and finally diluting and removing any remaining vapor by aeration[4]. For each of these phases, three variables must be quantified:

- Air flow rate
- Time
- Concentration

H_2O_2 concentration is difficult to measure directly. In these tests we calculated an average concentration. The calculation was based on the known internal volume of the cabinet, the known flow rate of vapor from the generator and the "no-load" decomposition rate for H_2O_2.

During the sterilization phase, the "exposure" must also be quantified. Exposure describes the total mass of H_2O_2 that comes into contact with the cabinet surfaces. It is a function of time and concentration. To measure exposure, we used indicator strips which turn color depending on the amount of H_2O_2 that has settled on their surfaces.

During the aeration phase, when the H_2O_2 is reduced to the threshold limit value of 1 PPM, we used a Drager tube to measure concentration. The tube location was established by tests with several Drager tubes to find the part of the cabinet with the least effective ventilation (the "worst case"). Then this single location was used for vapor sampling during the aeration phase. Samples were taken every hour during the first part of aeration, then at half hour intervals as the concentration approached 1 PPM.

After the cycle parameters were established, their decontamination effectiveness was verified by five consecutive growth tests using B. stearothermophilus[5]. In each test, ten stainless steel coupons were inoculated with 1 x 10^6 spores. The coupons were placed throughout the cabinet in locations shown in figure 3. After each cycle, the coupons were aseptically removed from the BSC and incubated in trypticase soy broth. An identical coupon that had not been through the decontamination cycle was incubated with each group of ten coupons as a control.

The broth in each tube was checked daily for growth. If no growth occurred after seven days, the coupon was considered decontaminated. Cycle parameters were considered valid when, after seven days, all 50 decontaminated coupons revealed no growth and at the same time, all five control coupons showed growth. Cycle parameters were adjusted and the testing repeated until this standard of validation was achieved.

To some extent, decontamination cycle parameters are a function of the capacity of the VHP™ 1000 generator and its operating characteristics. In this research, we used the maximum generator flow rate of 12 cubic feet per minute (cfm) to keep the cycle time to a minimum. Likewise, the AMSCO cycle development guide provided the values we used for the targeted concentration levels for each phase.

RESULTS

Of the materials tested, all proved structurally compatible except for open-celled, black neoprene gaskets and the nylon guides for the cabinet window. After several hundred cycles the nylon became brittle, and the neoprene gasket became soft and lost its shape. Concerning the issue of undesirable absorption of H_2O_2, only the HEPA filter frames absorbed enough to cause problems in the aeration phase. Outgassing of absorbed vapor from the filter frames extended the time required to reduce H_2O_2 concentration. Consequently, the initial filters were replaced with aluminum-frame units. Apart from these observations, the the cabinet materials appeared unchanged even after several hundred cycles of exposure to H_2O_2 vapor.

Optimizing vapor distribution required several iterations. Initial tests showed that vapor distribution was not effective. This was demonstrated by the time required for the first exposure indicator to change color and the lag time before the last indicator changed to match. In the supply plenum, the indicator changed within five minutes. But above the exhaust filter, ninety minutes were required before the indicator showed the appropriate exposure had been reached. To improve this poor distribution, an additional flow channel was installed, connecting the plenum above the exhaust filter to the supply blower plenum. A subsequent test showed this modification to be effective. Full exposure was achieved in all parts of the cabinet within times ranging from 13 to 30 minutes.

Following this modification, we began tests to determine the shortest effective time for each phase of the cycle. If the aeration phase took longer than five hours to reduce H_2O_2 vapor concentration to 1 PPM, we reduced the injection rate (H_2O_2 mass flow rate) during the sterilization phase. However, as the injection rate was reduced, it took longer to build full concentration above the filter. That problem prompted us to enlarge the opening we had made between the filter plenum and the supply blower plenum. The larger opening improved the distribution of vapor, allowing us to reduce the aeration phase by an additional two hours.

Table 4 shows the final values for air flow rates, H_2O_2 mass flow rates and exposure times. The spore growth test procedure showed that in all five runs with these parameters, there was no growth of microorganisms on any of the 50 test coupons.

Phase I - Dehumidification		
	Air Flow Rate	12 cfm
	Time	60 minutes
	Absolute Humidity	2.3 mg/l
	Relative Humidity	10%
Phase II - Concentration		
	Air Flow Rate	12 cfm
	Time	1 minute
	H_2O_2 Flow Rate	6.7 gr/min
Phase III - Sterilization		
	Air Flow Rate	12 cfm
	Time	30 minutes
	H_2O_2 Flow Rate	3.0 gr/min
	H_2O_2 Concentration	2.75 mg/l
Phase IV - Aeration		
	Air Flow Rate	12 cfm
	Time	210 minutes
	Final Concentration	≤1 PPM

Table 4. H_2O_2 decontamination cycle parameters for the Baker Model SG-600 biological safety cabinet. These were developed using AMSCO guidelines.

DISCUSSION

H_2O_2 vapor decontamination can be an effective alternative to formaldehyde and ethylene oxide, but only if appropriate cycle parameters have been developed and materials checked for H_2O_2 compatibility. Each BSC model is different, so each must go through these procedures .

When establishing the cycle parameters and making modifications to assure even vapor distribution, it is especially important to ensure that none of the Class II features are compromised. This means that any new penetrations must be sealed, all materials must be tested for frequent exposure to H_2O_2 vapor and the operational air flow characteristics of the cabinet must remain undisturbed. Changes to these features may affect cabinet performance, and therefore operator safety.

Several useful observations were made during this project, beginning with the critical fact that total cycle time depends primarily on the unique air flow pattern within a given cabinet. If air distribution is very even, concentration can be reached quickly.

DISCUSSION..... Continued

If concentration is reached quickly, then the total amount of H_2O_2 needed for sterilization is low. If the total mass flow of vapor is low, the cabinet can be quickly purged of residual vapor during the aeration phase (which is the longest part of the cycle).

Shortening the cycle time is beneficial in several ways: less H_2O_2 is used, the cabinet downtime is shorter and the cabinet materials are exposed to the corrosive effects of H_2O_2 vapor for shorter periods, so maintenance is reduced.

Another important observation is that materials which react unfavorably with H_2O_2 must be replaced. Failure to substitute compatible materials may triple or quadruple the time needed for decontamination and reduce the Class II integrity of the cabinet.

Conversely, when air circulation patterns are optimized and compatible materials used in cabinet construction as accomplished during this project, the benefits of H_2O_2 vapor decontamination are greatly enhanced.

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