Using Hydrogen Peroxide Vapor To Decontaminate Biological Safety Cabinets

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ABSTRACT
Recent research has shown that hydrogen peroxide vapor (H₂O₂) can be used to decontaminate biological safety cabinets (BSC’s) as an alternative to using formaldehyde or ethylene oxide. H₂O₂ is non-carcinogenic, highly effective as a decontaminant and is environmentally benign. However, H₂O₂ vapor decomposes quickly, so the gas must be rapidly circulated throughout the BSC. Also, hydrogen peroxide vapor attacks some materials. Consequently, existing cabinets need physical changes and material substitutions so the potential advantages of H₂O₂ can be fully realized.

HYDROGEN PEROXIDE DECONTAMINATION COMPARED TO OTHER METHODS
Ethylene oxide gas and formaldehyde have traditionally been used to decontaminate BSC’s. Both compounds are carcinogenic. Their vapors must be neutralized before being vented to the atmosphere, and they can present hazards during clean up operations. H₂O₂, in contrast, is not carcinogenic. The gas breaks down quickly into oxygen and water, and the decontamination procedure is a self-contained and automated process.

The exact decontamination reactions involving H₂O₂ are not well understood, but researchers hypothesize that the active component is the oxygen radical released as the H₂O₂ decomposes. Studies performed on a wide variety of fungi, bacteria and viruses show that D-values—which indicate the length of time needed to kill a specific concentration of organisms—are quite favorable with H₂O₂.

DECONTAMINATION PROCEDURE
H₂O₂ decontamination requires four steps: drying the cabinet, concentrating the vapor, sterilizing the cabinet and finally diluting and removing any remaining vapor by ventilation.

Before sterilization, humidity inside the cabinet must be removed so the air can absorb H₂O₂. In air, hydrogen peroxide vapor behaves much like water vapor (H₂O). Consequently, if the relative humidity is high inside the cabinet, the air will not have enough capacity to absorb the amount of hydrogen peroxide needed for effective decontamination. This is why a VHP™ 1000 generator begins its cycle by circulating dry air through the cabinet to remove existing water vapor.

After drying the cabinet, hydrogen peroxide is introduced at a high injection rate until the air is nearly saturated. At this concentration, organisms contacted by the vapor will be killed within two to three minutes based on D-values, but the vapor must be carried to all parts of the enclosure. Tests on typical cabinets have shown that the sterilization phase should last from 25 to 90 minutes, depending on the cabinet characteristics and on the uniformity of vapor distribution.

After full concentration is reached, the generator reduces the injection rate, but it continues to supply vapor to make up for the steady decomposition of H₂O₂ to oxygen and water. It is important to maintain a steady, nearly saturated level of vapor to ensure adequate decontamination—commonly defined as killing at least 1 x 10⁶ organisms. After the sterilization phase is complete, the generator removes the air at a rate of 20 cubic feet per minute (cfm).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Treatment</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td>5 to 7</td>
<td>H₂O₂ Vapor (Factory-made for VHP™, air leaving @ 1 PPM)</td>
<td></td>
</tr>
<tr>
<td>5 to 8</td>
<td>Formaldehyde</td>
<td></td>
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<tr>
<td>9 - 12</td>
<td>VHP™ (Field-modified, air leaving @ 2 PPM)</td>
<td></td>
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<tr>
<td>27 to 32</td>
<td>VHP™ (Field-modified, air leaving @ 1 PPM)</td>
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Figure 1. Comparing decontamination methods on the basis of hours required to kill 1 x 10⁶ organisms

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and breaks down any H₂O₂ it captures. Then the generator dehumidifies the air and returns it to the cabinet. This ventilation process continues until the vapor concentration falls to about 1 PPM.

**VAPOR DISTRIBUTION**

When formaldehyde or ethylene oxide are used for decontamination, the vapors are distributed partly by diffusion. Since these vapors are persistent, and since hours rather than minutes are needed for formaldehyde to kill enough organisms for full decontamination, this relatively slow distribution poses no problem. H₂O₂ vapor, in contrast, decomposes within a few minutes, which means there is not enough time for diffusion to carry the vapor to all parts of the cabinet. This is an important issue. When BSC’s are sealed to isolate them for decontamination, there are “dead-end” plenums inside the enclosure where air does not circulate freely. To eliminate these dead spots and effectively decontaminate all surfaces, the H₂O₂ must be actively distributed throughout the enclosure. It is best to use the cabinet fan for this purpose.

While VHP™ 1000 generators use a fan to supply the vapor to the cabinet, that fan does not normally have enough capacity to accomplish uniform distribution. For example, a typical generator may circulate 20 cfm, compared to the cabinet fan which can move between 450 and 650 cfm. This mismatch of fan capacities creates a sharp concentration gradient between different sections of the cabinet. Unless specific provisions are made to supplement the generator circulation capacity, the sterilization process takes two or three times longer, a result which most users of BSC’s would prefer to avoid.

Decontamination cycle time increases when vapor distribution is not uniform. In theory, if the H₂O₂ could be instantly and uniformly distributed throughout all corners of the cabinet, the sterilization phase would only require a few minutes—all surfaces and all organisms would be contacted simultaneously with vapor at full concentration, and very fast kill rates would be possible. Cabinet geometry, however, prevents instant, uniform vapor distribution, so more time is needed to build up full concentration in all corners of the enclosure. Decontamination time generally takes about an hour rather than a few minutes for this reason. Specific cabinet designs can either shorten or lengthen this time. For example, cabinets often have “dead-end” plenums with little air circulation so vapor cannot penetrate rapidly at full concentration. The easily-accessed part of such cabinets may be sterilized within a few minutes, but the dead-end plenums may not receive fully-concentrated vapor for much longer, because the H₂O₂ decomposes before it can reach into all corners.

Extended sterilization times lead to a second problem. In the long time it takes for vapor to reach into dead-end plenums, the more easily-accessed surfaces are exposed to full-strength H₂O₂ for a much longer period. Their surfaces may adsorb vapors which must be removed in the ventilation phase, extending that part of the decontamination cycle as well.

Consequently, vapor distribution uniformity and speed are important issues. For cabinets currently in use, modifications are necessary to achieve rapid, uniform distribution. Otherwise some of the theoretical improvements offered by H₂O₂ may not be achieved in practice, and total decontamination cycle time can be greatly extended.

**MATERIAL COMPATIBILITY**

Many materials are unaffected by hydrogen peroxide vapor, but others can absorb the gas and still others are attacked. For example, most HEPA filter frames are made of cellulosic material, which absorbs H₂O₂ at high concentrations, and then releases the gas slowly. Outgassing can continue for several days, depending on how the hydrogen peroxide reacts to the particular environment. This means the gas can build up—in low concentrations—in the work area and in the lab if the cabinet is not vented to the outside.

Nylons are an example of materials which are attacked by the vapor, and some nylons are used in electrical connectors and other electrical components. Similarly, some neoprenes are degraded by H₂O₂, which means that existing gaskets may have to be replaced with a different material. A recent test of two gaskets suggests that neoprene may degrade in proportion to its exposed surface area. The dense gasket material lasted for many more decontamination cycles than an open-celled gasket made of similar material.

Breakdown of either electrical components or gaskets can be detrimental to safety, as well as adding maintenance expense and causing costly equipment down time. If the institution is considering the retrofit of existing equipment to use H₂O₂ vapor decontamination, cabinet materials should be identified and tested for compatibility.

Unfortunately, compatibility can only be established by exposing the material to hydrogen peroxide vapor. Just as liquid hydrogen peroxide cannot be used to predict sterilization performance of its vapor, liquid H₂O₂ cannot reliably predict degradation caused by the vapor nor the absorption behavior of materials when exposed to the gas.

**DIFFICULTIES WITH H₂O₂ IN EXISTING CABINETS OR RETROFIT KITS**

Existing cabinets can be modified in the field to accept hydrogen peroxide vapor generators, or standard cabinets provided with retrofit “kits”. However, these alternatives are less than optimal because:

1. Such cabinets are not designed for fast distribution of H₂O₂, and poor vapor distribution will require long cycle times—considerably longer than formaldehyde cycles.
2. Without material compatibility testing, important components may degrade unacceptably.
3. Unless cellulosic materials are replaced or treated they adsorb H₂O₂, releasing it slowly during the ventilation phase. This slow release often explains why it may take 15 to 24 hours of ventilation to reduce the vapor concentration to 1 PPM.
RETROFIT...Continued

4. Penetrations needed to connect VHP™1000 generators may void U.L., NSF or CSA certification.
5. It may be difficult to seal the cabinet to avoid vapor leaks, and informally-designed couplings may disconnect if bumped by custodial personnel or inexperienced workers who may not be fully aware of the potential hazards presented by H₂O₂ leaking into the lab environment.
6. If the cabinet blower is used to recirculate the air, there is the danger of inadvertently pressurizing the cabinet work area, causing plastic sheets and tape to blow off unexpectedly.

CABINETS SPECIFICALLY DESIGNED FOR HYDROGEN PEROXIDE VAPOR DECONTAMINATION

The end user may elect to purchase a cabinet specifically designed for H₂O₂ vapor decontamination. This alternative offers several potential advantages:
1. The manufacturer (rather than the end-user) is responsible for design changes which ensure successful results.
2. Eliminating dead spots and avoiding vapor adsorption by cellulosic materials achieves a cycle time of approximately 6 hours or less.
3. Changing H₂O₂-sensitive materials allows more cycles without degradation.
4. Replacing cellulosic materials avoids slow outgassing and the potential for vapor build-up in the work space and in the lab.
5. Carefully considered design details allow predictable cycle times and reliable procedures.
6. H₂O₂-related safety features are included in the cabinet design.
7. UL, NSF, and CSA certification can be maintained.
8. Sealing mechanisms, integral to the cabinet design, are more effective and reliable than ad-hoc modifications.
9. The manufacturer provides documentation supporting the effectiveness of decontamination cycle times and procedures. This can be useful in validating a process and in assuring control of critical variables in research activities.

REFERENCES


VHP is a trademark of AMSCO, Pittsburgh, PA.

SUMMARY

H₂O₂ vapor decontamination offers safety and environmental improvements over formaldehyde. However, to achieve these advantages in existing equipment, extensive modifications are necessary to ensure that the gas is quickly and uniformly distributed to all parts of the cabinet. Also, it may be necessary to change some materials to avoid premature degradation and vapor absorption. New cabinets specifically designed for H₂O₂ decontamination are a better alternative, as they provide a number of useful benefits in addition to faster and more predictable decontamination cycle times than retrofitted cabinets.
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