Overcoming Limitations of Vaporized Hydrogen Peroxide

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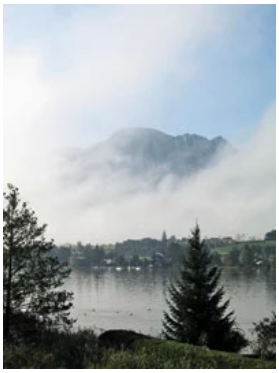
Vaporous hydrogen peroxide, used for sterilization and decontamination, is highly potent but presents implementation challenges.

The use of hydrogen peroxide (H_2O_2) in the global healthcare industry and other industries that require high levels of contamination control has grown steadily. This growth is attributable to the chemical's ability to kill spores and sterilize materials, which has been demonstrated in a variety of practical applications. Properly used, H_2O_2 is an effective sterilant capable of efficient and rapid elimination of contaminating microbes. Some difficulties have been associated with the implementation of H_2O_2 processes in the healthcare field although these issues appear to have been avoided in commercially sterile food and beverage manufacture. Specifically, persistent problems regarding the development of H_2O_2 processes and their subsequent validation have been reported. The author discusses the technical issues associated with achieving lethal concentrations of H_2O_2 delivered in vaporous form on decontamination targets, explores the core scientific principles behind H_2O_2's use in decontamination and sterilization, and provides experience-based solutions to frequently encountered operational issues.

Hydrogen peroxide (H_2O_2) is an extremely powerful oxidant that is capable of effectively killing resistant spore-forming bacteria over a wide range of concentrations; at concentrations of 3% or less, it is suitable for use as a topical antiseptic (1). H_2O_2 has been accepted by both FDA and the US Environmental Protection Agency (EPA) as a sterilizing agent for many years (2, 3). In the food industry, H_2O_2 is widely used to sterilize containers, closures, and aseptic chambers (i.e., isolators) used for manufacturing low-acid and dairy-based beverages as well as other applications (4).

The potency of H_2O_2 as a sterilant and its usefulness in a broad range of antimicrobial applications are beyond dispute. The problems associated with vaporized H_2O_2 processes in the healthcare industry lie in fundamental misunderstandings concerning physicochemical characteristics of H_2O_2 sterilization. These errors profoundly influence real-world H_2O_2applications.



What is Vapor?

There are three primary states of matter--solid, liquid, and gas. The term "vapor" is defined in several ways. Scientifically, a vapor is a gas at a temperature lower than its critical point; a vapor is a gas phase where the same substance can also exist as a liquid. An example is atmospheric water vapor. At temperatures above the dew point, water in the atmosphere is a gas. As the temperature is lowered through the dew point, the gaseous water condenses to form a fog or mist, or it can condense and form liquid water on a cold surface. Another definition of vapor is visible moisture in the air, as in fog or steam—a system in which a liquid is suspended in a gas.

Figure 1 shows water in various phases: the lake, the dense fog at the foot of the mountain, the wisps of cloud,

The density of the fog or cloud varies with its temperature. It is thickest (i.e., suspending the most liquid) near the base of the mountain where it is coldest. It is clearly less dense, with less suspended water droplets near the top of the image where the temperature is higher.

One of the major difficulties with hydrogen-peroxide (H_2O_2) processes is the use of a vapor for delivery of H_2O_2 and water (H_2O) to the target chamber. It must be understood that a vapor is a mixture of air and liquid that is present within the chamber. In decontamination or sterilization using H_2O_2, the liquid phase is comprised of both H_2O_2 and H_2O, and the concentration of each in the gas and suspended liquid state can vary across the system.

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Understanding vapors

To fully understand the physical factors that affect the distribution of H_2O_2 in the vapor phase, one must consider the factors that affect vapors in general and the factors that allow them to exist in air, which is the medium in which H_2O_2 in the vapor phase is distributed within a decontamination target. Air contains varying, but small, amounts of water in the vapor phase, which is described using the term relative humidity (RH). An important factor in the distribution of a chemical is the dew point. The dew point is, in simplest terms, a function of both concentration and temperature. When the concentration of water exceeds the saturation point at a particular temperature, condensation occurs. The gaseous water converts to the liquid phase, and droplets of liquid water may appear. On the other hand, if the water concentration is below the saturation point, it will remain in the gas phase. When the temperature of the air is actively lowered (or simply drops as a function of thermodynamics) below the dew point, some portion of the water (H_2O) present as a gas mixed with air condenses and forms liquid droplets. We observe this as clouds, dew, fog, or frost.

The typical H_2O_2 process

The process that most H_2O_2 generator and isolator manufacturers use for H_2O_2 introduction is one in which a hot air stream is used to introduce a heated H_2O_2/H_2O gas into the target environment, which may be an aseptic chamber or isolator. Within the generator, the temperature of the air/H_2O_2/H_2O mixture is sufficiently high that all three materials are in a gaseous state. The hot air is conventionally at temperatures in excess of 100 °C, which takes advantage of the respective boiling points of the pure components (i.e., H_2O = 100 °C, H_2O_2 = 150.2 °C, and a 30-35% aqueous solution of H_2O_2 = approximately 108 °C). At these temperatures, both H_2O_2 and H_2O are present as gases and are carried into the target vessel with the hot air. The H_2O_2/H_2O is supplied as an aqueous solution of H_2O_2in varying percentages typically ranging from 31% to 50% H_2O_2. At typical room temperatures, each of these solutions is predominantly liquid, and the headspace air within the closed containers has a small amount of gas phase H_2O_2/H_2O that is in equilibrium with the liquid.

If the concentration remains below the saturation point upon introduction into the target environment, then both the H_2O_2 and H_2O will remain in the gas phase. When the hot and relatively humid gas mixture from a H_2O_2 generator is introduced to the target chamber, it will encounter colder air as well as ambient temperature surfaces of the chamber and materials inside it. As the hot gas mixture cools to the temperature of the chamber, it will fall below the dew-point temperature of both H_2O_2/H_2O, and some portion of these materials will condense on the surfaces as liquids. In effect, the H_2O_2/H_2O are returning to their initial equilibrium state of liquids in equilibrium with the adjacent gas, which they possessed before being converted to a gas in the generator.

Condensation that forms on the surfaces will tend to be nonuniform in concentration across the chamber for several reasons:

- The H_2O_2 will condense first due to its lower equilibrium vapor pressure (i.e., lower dew point) relative to H_2O.
- The temperature in the system may be non-uniform across the chamber and is generally hottest near the inlet where the hot gas mixture is introduced; for the purposes of vapor-phase hydrogen peroxide (VPHP) technology, ± 2.5 °C can be considered effectively uniform.
- The continued introduction of the hot gas mixture into the chamber, in which VPHP generators rely on continuous replenishment of mixture vapor, results in a slow increase in temperature within the chamber. This effect is more pronounced in smaller enclosures and those with relatively low mass.
- In larger enclosures, the amount of heat added by the hot air stream laden with H_2O_2/H_2O will have little impact on temperatures remote from the injection port.
- Where the localized temperature within the enclosure is low enough and concentrations of H_2O_2 and H_2O are high enough, they will condense. Many present-day H_2O_2 generator systems are designed such that the process relies on the presence of condensation. In these cases, one should recognize that the

condensate on surfaces.

- Depending upon the decontamination approach used, H_2O_2/H_2O introduction during the process dwell period can be continuous, intermittent, or absent entirely. In cases where the hot air/vapor stream is present only during a comparatively short initial introduction period, the effects of the hot air stream on target chamber temperatures will be less profound.
- Chambers with a large number of objects to be decontaminated have added surfaces upon which condensate may accumulate. As the load size increases, the amount of H_2O_2 added and/or the process dwell period may need to be increased to ensure condensation on all target surfaces.

The extent of condensation that occurs depends upon the temperature (i.e., colder locations will have more condensation), the concentration or amount of H_2O_2/H_2O introduced (and removed if a circulating process is used), the size of the enclosure (i.e., affects the surface/volume ratio), and the quantity of material within the chamber (i.e., adds to the surface area).

Phase states in the enclosure

In must be understood that the enclosure will contain a mixture of air/H_2O_2/H_2O internally, with some of the H_2O_2/H_2O in a liquid state on surfaces and the remainder in the gas phase. There is no simple means to establish how much H_2O_2/H_2O is in each phase or where in the chamber a particular phase is present. Additionally one cannot know the percentage of H_2O_2 or H_2O at any single location, and certainly not at every location within the enclosure. The Gibbs Phase rule makes it clear that conditions can vary across the system (see **Equation 1**).

$$F = C - P + 2 = 3 - 2 + 2 = 3 \quad \text{(Eq. 1)}$$

where F = number of degrees of freedom (i.e., concentration, temperature, pressure), C = number of components in the system, and P = number of phases in the system.

Almost nothing is known with certainty with respect to concentration and location. There is, however, one constant in the process: H_2O_2 is lethal to microorganisms in both the gas and liquid phases. It is reasonable to assume that liquid-phase kill will be somewhat faster than the gas-phase kill for two important reasons as further outlined:

- The concentration of H_2O_2 in the liquid phase will always be higher. A 35% H_2O_2 mixture will have equilibrium concentrations of H_2O_2 of ~2% in the gas phase and ~79% in the liquid phase (5).
- The presence of adequate moisture at the point of sterilization is certain in liquids, as H_2O is the other component of the liquid phase.

An older reference describes more rapid kill occurring with H_2O_2 in a gas-phase process compared to a liquid-phase process (6). This reference identifies a gas-phase process at 25 °C, with no mention of any liquid H_2O_2 present. At that temperature, however, H_2O_2 is a liquid, so there must be some liquid H_2O_2 in equilibrium with the gas. There is no means to establish that the kill in this “gas” process was actually accomplished in that phase. It is more likely that the cited kill was accomplished in the liquid phase. Misinterpreting what is actually “vapor” as a “gas” has led to the erroneous belief that gaseous-phase kill is more rapid than liquid-phase kill.

The expected microbial kill rates in the system might appear as shown in **Figure 1**, which visualizes H_2O_2 sterilization as a process that occurs within a band, bounded by the extremes of liquid and gas-phase kill. **Figure 1** represents what is believed to occur and does not reflect any specific H_2O_2 process. The absolute slopes of the death curves are unknown. Given that the localized concentrations in both phases are variable due to temperature differences and proximity to the inlet with its heated air supply, it must be recognized that there will be different kill rates in different locations in both the liquid and gas phases. **Figure 1** represents what might occur at a single point within the chamber; similar appearing death curves with differing slopes can be considered for other locations where the local conditions are different. These variations are the underlying cause of the variable performance experienced when using vapor-phase H_2O_2 as a lethal process.

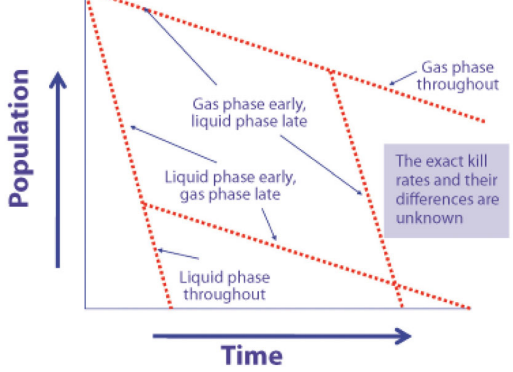

Figure 1: Estimated relative kill rates in liquid and gas phases; the exact kill rates and their differences are unknown. (Figure courtesy of the authors.)

D-values for H_2O_2 decontamination

The death curves in **Figure 1** seem to show that a D-value (or an approximation of one) could be established against a challenge microorganism for the combined processes. That assumption is faulty because there is no way of establishing what conditions (e.g., phase, concentration, or humidity) are present in the system at the point where the microorganism is killed. D-value determination requires knowledge of the specific lethal conditions to which a microorganism is exposed. In a single-phase sterilization process, gas or liquid, information on concentration of the agent, humidity (assumed at 100% for liquid processes), and temperature is readily determined. In the context of H_2O_2, this is easiest for liquids, and published D-values for Geobacillus stearothermophilus in various H_2O_2/H_2O liquid solutions are available (1). These liquid phase D-values demonstrate extremely rapid kill (in seconds) at even modest H_2O_2 concentrations (7). At the estimated concentrations where condensation first occurs in vapor H_2O_2 processes, the D-values should be lower as the concentration will be substantially higher than that published in the literature. Unfortunately, no comparable data are available on H_2O_2, where a strictly gas-phase process is present. Thus, any labeled "D-values" for vapor H_2O_2 biological indicators must be considered nothing more than an approximation as the killing conditions are unknown. The conditions of kill may be consistent enough that they could be replicated in an independent study in the same test system. What cannot be established from these labeled "D-values" is how that same biological indicator will respond in a different environment where the conditions are also unknown and most likely substantially different.

In the 20-plus years that this industry has been using H_2O_2 decontamination, a BIER (biological indicator evaluation resistometer) vessel for H_2O_2 has not been developed as a standard for compendial or routine use. The same conundrum faced with respect to variable and unknown biphasic conditions in a larger system has prevented the development of a H_2O_2 BIER. The absence of a BIER vessel and, thus, a fully useable "D-value" for H_2O_2 biological indicators has caused some difficulties. What can be established from the vendor "D-value" is the relative resistance of one lot to another from the same vendor. How any individual lot will perform under different conditions is something the user must determine for each application.

One suggested approach to get beyond this lack of a definitive D-value for a biological indicator is to establish a process or system "D-value" for a biological indicator within a large enclosure and rely upon that as the basis for destruction in the system rather than the vendor's reported value. This approach presumes that the conditions used to establish the process/system "D-value" are representative of the entire system. That assumption is decidedly not the case, nor is it known whether the location(s) chosen for the process "D-value" determination are best case or worst case with respect to kill across the chamber. A number, which is not a D-value in the strict sense, can be calculated, but the utility of that number in any estimation kill rate across the chamber is essentially nil.

Reports of vapor-phase "D-value" variations as a consequence of different substrates must also be recognized as uncertain (8, 9). Because there is no objective biological indicator evaluation method available, published "D-values" are not standardized and thus of very limited use. Unless the concentration on the individual surfaces tested can be known and demonstrated to be constant, any hint that the substrate variations are meaningful must be viewed with some skepticism. There is also some published evidence that "D-values" may vary with spore concentration applied to the carrier material, which means kill may not be linear with concentration. That represents a serious flaw in the use of any biological indicator.

Is safety a concern with H_2O_2?

Given the rapid kill observed in the H_2O_2 liquid phase, the difficulties in attaining consistent kill with H_2O_2 vapor processes can only be explained by a lack of adequate condensation, for there is little doubt then when

integrated into enclosures, rely on condensation to decontaminate/sterilize extremely rapidly.

Since the rapid kill provided by liquid H_2O_2 is well documented, why has industry been cautioned to avoid condensation in vapor H_2O_2 processes? The answer lies in the early teachings of AMSCO (now Steris) when the first H_2O_2 generator was introduced in the late 1980s. Caution was routinely raised regarding the potential hazards of high concentrations of liquid H_2O_2. (The H_2O_2 concentration in the gas phase at ambient temperature will always be substantially lower than its equilibrium concentration in the liquid phase.) The relevant safety issues with the use of H_2O_2 vapors are:

- *Explosive vapors.* The caution here relates to concentrations of > 70% H_2O_2 giving off explosive vapors at temperatures greater than 70 °C (11). If this situation were to occur anywhere in vapor processes, the generators themselves would represent the greatest risk. Temperatures inside enclosures rarely exceed 30 °C, and thus the likelihood of this presenting a real-world problem during a sterilization process is unlikely.

- *Hazardous reactions.* There are reports of H_2O_2 reacting with greases, alcohols, ketones, carboxylic acids (particularly acetic acid), amines, and phosphorus. Small amounts of other materials that contain catalysts (e.g., silver, lead, copper, chromium, mercury, and iron oxide rust) can cause rapid decomposition and an explosive pressure rupture of the containing vessel if it is not properly vented (12). None of these compounds and materials is typically present in pharmaceutical enclosures.

- *Corrosivity.* This is possible with some materials, but the typical stainless steel, glass, and other materials exposed to H_2O_2 are known to be compatible and are chosen explicitly for that purpose. The chemical compatibility of H_2O_2/H_2O solutions is well documented.

- *Worker safety.* The US Occupational Safety and Health Administration has established an 8-hour, time-weighted average for exposure to H_2O_2 of 1 ppm, with an immediate hazard in the presence of concentrations greater than 75 ppm (13, 14). This limit is managed in pharmaceutical facilities through external alarms in the surrounding areas and requirements for aeration before personnel or material exposure.

While there is a need for caution with respect to the use of vapor phase H_2O_2, undue concern is unwarranted. In more than 20 years of use in the global industry, there have been no reported incidents of personal injury or equipment damage associated with this process.

Claims that vapor-phase H_2O_2 processes do not result in condensation are speculative. The laws of physics and temperature within enclosures are such that some measure of condensation will always occur, and in many recent equipment and process designs the creation of condensation is intentional. Thus, within the context of real-world experience, the safety issues associated with vapor H_2O_2 systems where condensation is present appear to be adequately managed, assuming appropriate worker-safety precautions are maintained.

Limitations of multipoint process-control measurements

FDA's *Guideline on Sterile Drug Products Produced by Aseptic Processing* recommends: "The uniform distribution of a defined concentration of decontaminating agent should also be evaluated as part of these studies" (15). This suggestion is made without reference to a specific methodology that could be employed. There is no technology that could address this expectation throughout a two-phase environment. Nor would the resulting data on concentration in the gas phase be useful in correlating to microbial kill on surfaces. When appropriate amounts of H_2O_2 are used for decontamination or sterilization, some of the available instruments, such as those that rely on near-infrared transmission, are unusable due to condensation on the lenses. Because accurate measurement is not possible, chemical indicators provide the only widely available means to confirm that H_2O_2 is, or was, present at a specific location.

Problems in an unsteady-state process

The introduction of H_2O_2 into a room-temperature enclosure uses vapor-process heating to convert the liquid solution into a gas for mixing and distribution in hot air. The temperatures in vaporizers are in the range of 105-150 °C. This high temperature results in some localized heating of the enclosure, primarily in locations close to the entry point of the heated materials. The effects of this heat input are multiple:

- Temperatures during the process will change over its duration with the greatest impact found in locations nearest the infeed locations. This heating is more pronounced in smaller, flexible-wall and lightly loaded enclosures where there is less overall mass.

- The resulting changes in temperature will result in varying amounts of condensation (and thus kill) across the enclosure (and also varying over the duration of the process dwell period at a single

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