This article presents research into factors relating to the efficiency and efficacy of vapor phase hydrogen peroxide decontamination. The studies presented demonstrate that initial dehumidification of the target can adversely affect efficacy and that continuous injection of vapor hydrogen peroxide is not required.

A New Approach to Vapor Hydrogen Peroxide Decontamination of Isolators and Cleanrooms

by Kunihiro Imai, Soma Watanabe, Yasusuke Oshima, Mamoru Kokubo, and Jim Akers

The decontamination of aseptic work environments has been practiced in the healthcare industry for many years. Until fairly recently, formaldehyde vapor was used widely for decontamination of cleanrooms, a practice that is now falling into disfavor because of the carcinogenic nature of formaldehyde. Additionally, formaldehyde, like many forms of gas or vapor decontamination, requires elevated humidity for maximum effectiveness.

Research on sporicidal agents that could work effectively at temperatures close to those maintained in typical cleanrooms has continued to be an area of active study. Some promising candidates have been advanced over the last 20+ years. The combination of peracetic acid and hydrogen peroxide, which is known to be somewhat synergistic, has been used for the decontamination of aseptic enclosures such as isolators for both sterility testing applications and in some production applications. Chlorine dioxide, a well known oxidizing gas also has been utilized for decontamination or sterilization at relatively cool temperatures. Also, ozone, which like Chlorine dioxide and Hydrogen peroxide is a powerful oxidant, has been advanced as a possible decontaminating agent for aseptic applications.

In the late 1970s, the sporicidal efficacy of vaporized hydrogen peroxide was first discovered. This technology was licensed by American Sterilizer Corporation (AMSCO) in 1980 from American Hospital Supply Corporation, and initial studies focused on the use of this technology in the sterilization of hospital equipment and supplies. However, by the late 1980s, the introduction of a new type of aseptic technology using relatively small remotely accessed enclosures emerged and was initially applied to the conduct of the sterility test. As isolator technology came into wide spread use for production and research activities, VHP rapidly became the method of choice for environmental decontamination.

Although the efficacy of VHP against microorganisms was well established, the development of VHP decontamination cycles and their subsequent validation proved to be a significant challenge. In the early years of isolator technology, some proponents considered the isolator an "absolute" or "sterile" enclosure, which could be capable of practical performance on par with terminal sterilization. This led to

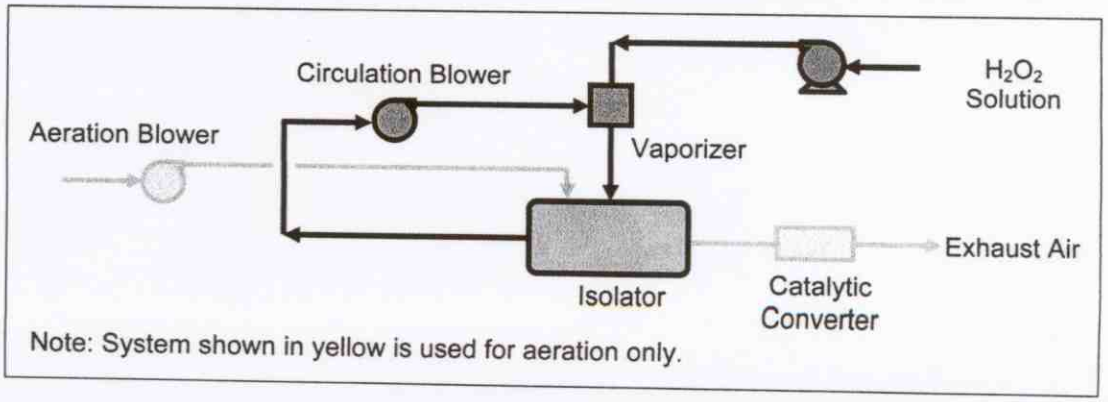

Figure 1. Process flow diagram.

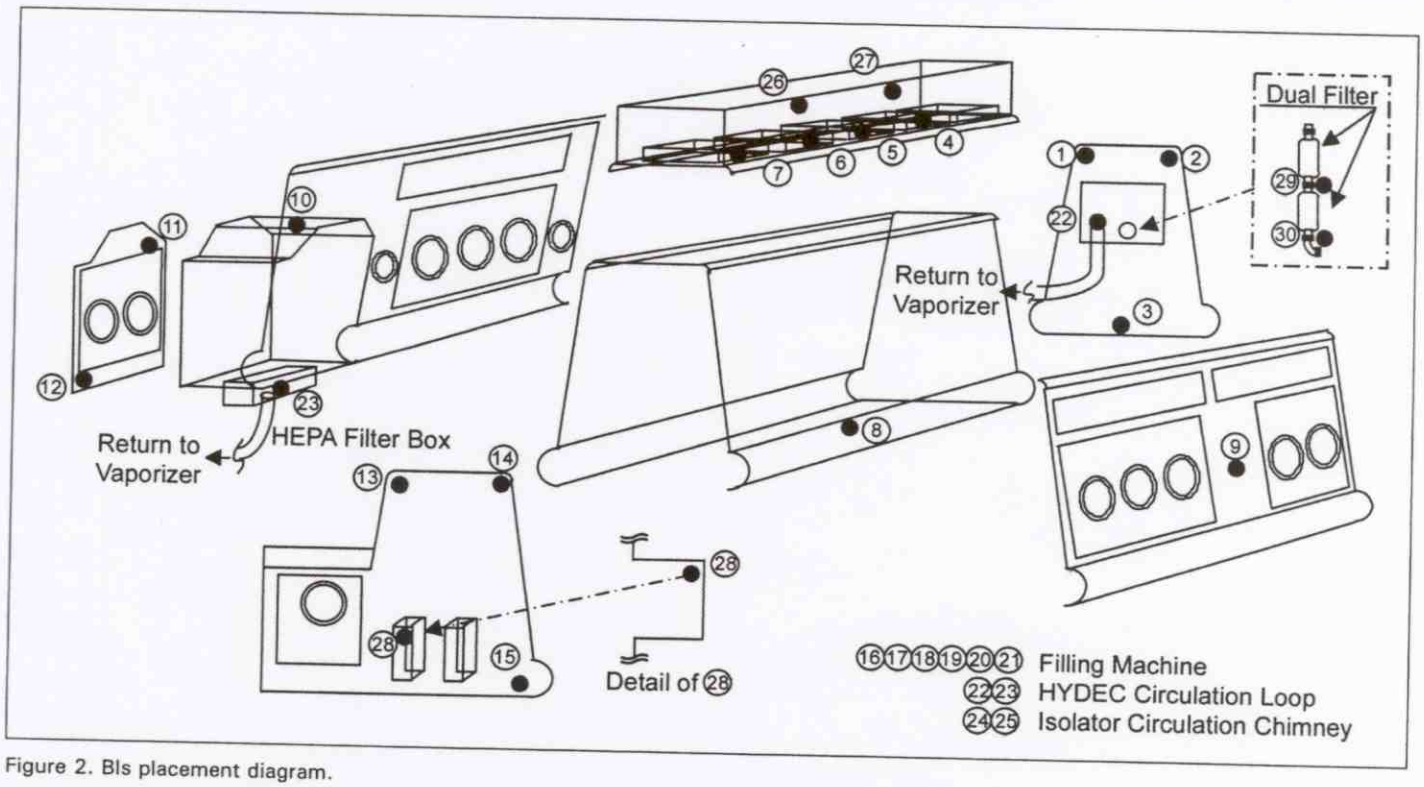

Figure 2. BIs placement diagram.

the notion that isolators must be sterilized, which then logically led to the accepted belief that validation of isolator "sterilization" with VHP should require a validation approach in terms of validation and process control similar to that of, for example, moist heat sterilization. After considerable debate, it was finally generally accepted that *decontamination* was a more reasonable target than *sterilization*. Nevertheless, emphasis on validation of VHP did not lessen the demand for process monitoring, most specifically involving the accurate measurement of vapor concentration emerged.

This article describes the development of a Vapor Phase Hydrogen Peroxide (VPHP) generation system with cycle design parameters that are much different than those which have been used in industry. The system described is based upon considerable research and development stemming from empirical experience in the design and validation of isolator systems. The authors believe that this process model has not only efficiency advantages, but also describes a process which is more in keeping with what has long been known regarding vapor and/or gaseous sterilization.

The experiments described in this article were designed to evaluate key process control elements that have been thought to be required for optimal performance of VPHP systems. The authors hypothesized that there may be little or no value in the removal of moisture from the target prior to exposure to H_2O_2. In fact, it has been established with other forms of gaseous sterilization using oxidants such as Ozone and Chlorine dioxide that moisture levels above 65% were necessary for optimal kill effectiveness. It is known that elevated humidity enhances the efficacy of both Ethylene Oxide gas and formaldehyde vapor.

The authors also sought to experimentally evaluate the need for continuous replenishment of H_2O_2 vapor since there have been indications that the half-life of H_2O_2 was sufficiently long that replenishment was unnecessary.

Another matter that the authors of this article also wished to evaluate was the use of concentration monitors, specifically how well the measured concentration correlated with rate of spore inactivation. Information from the Steris cycle development guide appeared to indicate that vapor concentration measured in free air within the enclosure should correlate directly with the rate of spore inactivation. However, data published by V. Sigwarth had indicated that relatively high rates of inactivation were possible with significantly lower concentrations of vapor. Also, the authors' previous experience had indicated that a linear relationship between concentration in free air at a fixed temperature and rate of inactivation might not exist.

The data presented in this report demonstrates that it is possible to eliminate the dehumidification phase of the process in nearly all cleanroom or isolator applications. These studies also indicate that it is not necessary to continuously inject vapor H_2O_2 throughout a cycle's exposure period.

Total VPHP Injected	100g	80g	60g	40g	20g
Starting Temperature	32.0°C	32.5°C	33.0°C	31.0°C	32.2°C
Survivors / No. BIs Tested	0 / 30	0 / 30	24 / 30	29 / 30	30 / 30
Isolator not temperature controlled. Positives / Total BIs after 7 day incubation					

Table A. Survival data - variation of total injection quantity (initial humidity- 5% RH).

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Injection Quantity	70% RH	60% RH	40% RH	20% RH	5% RH
100g			0 / 30		
80g	0 / 30	0 / 30	0 / 30	0 / 30	0 / 30
60g	22 / 30	6 / 30	1 / 30	3 / 30	17 / 30
40g	28 / 30	27 / 30	13 / 30	26 / 30	28 / 30
Positives / Total BIs after 7 day incubation					

Table B. Injection quantity vs. RH in isolator - initial study temperature 20°C.

Materials and Methods

The vapor phase hydrogen peroxide generator described in this study operates at a fixed VPHP injection rate of 20g/minute. A process flow diagram of the generator as used in conjunction with an isolator target is shown in Figure 1. Two types of studies are reported in this communication. A number of experiments were conducted in a 6.5m³ aseptic filling isolator. The isolator is of a rigid wall unidirectional airflow design and encloses a vial filling system capable of 300 units/minute. The isolator and enclosed equipment are constructed primarily of 304 stainless steel and the isolator glazing is polycarbonate. Aeration was accomplished by direct venting at flow rates stipulated in the experimental data. The studies described herein were carried out at Shibuya's Morimoto factory in Kanazawa, Japan. A further study was conducted in a pharmaceutical cleanroom with an enclosed volume of 75m³ provided courtesy of Sankyo Pharmaceutical in Tokyo, Japan. VPHP was injected directly into the room environment using a generator. Freestanding circulation fans were employed to enhance the distribution of the vapor. A standalone aeration unit equipped with a catalytic converter accomplished aeration.

Biological Indicators (BIs) were sourced from Apex Laboratories of Apex, NC, US. The BIs consisted of *Geobacillus stearothermphilus* ATCC #12980 spores inoculated onto stainless steel substrates. The BIs were packaged in Tyvek envelopes. These BIs were chosen because they are representative of BIs most commonly used for cycle development and validation studies in vapor phase hydrogen peroxide applications. The nominal spore concentration per BI was approximately 2.0×10^6. The estimated population and nominal D-value are given in each results table where relevant. The BIs were cultured after each test in Soybean Casein Digest Agar and incubated at a temperature of 55°C for at least seven days.

Concentration of H_2O_2 vapor in all studies was done using a Draeger Polytron II. Low levels of H_2O_2 during and post-aeration (1-3ppm) were measured using Draeger test tubes.

Results

Experiments were conducted to determine whether vaporized H_2O_2 of a volume sufficient to achieve decontamination could be delivered to an isolator target rather quickly without the need for continuous replenishment throughout an exposure period. In this study, no conditioning phase was used and H_2O_2 was vaporized and injected over a period of only a few minutes. After completion of the injection phase, dwell periods of various lengths were tested during which the recirculation blower of the isolator operated. In the study shown in Table A, the isolator was initially dehumidified to 5% RH and various injection quantities were tested. A rather sharp break point can be observed between 60g of total injection in which 24 out of 30 BIs were positive for growth and 80g which resulted in no survivors. The injection rate of

Injection Quantity	RH (%)	10	20	30	40	50
40g	#1		6 / 6	4 / 6	5 / 6	2 / 6
	#2		6 / 6	6 / 6	5 / 6	2 / 6
	#3		6 / 6	6 / 6	6 / 6	4 / 6
45g	#1	6 / 6	4 / 6	0 / 6	0 / 6	0 / 6
	#2	6 / 6	5 / 6	1 / 6	0 / 6	0 / 6
	#3	6 / 6	6 / 6	1 / 6	1 / 6	0 / 6
49.5g (45x1.1)	#1	6 / 6	2 / 6	0 / 6	1 / 6	0 / 6
	#2	6 / 6	6 / 6	0 / 6	0 / 6	0 / 6
	#3	6 / 6	4 / 6	0 / 6	0 / 6	1 / 6
54g (45x1.2)	#1	5 / 6	2 / 6	0 / 6	0 / 6	0 / 6
	#2	6 / 6	0 / 6	0 / 6	0 / 6	0 / 6
	#3	6 / 6	1 / 6	0 / 6	0 / 6	0 / 6
58.5g (45x1.3)	#1	0 / 6	0 / 6	0 / 6		
	#2	1 / 6	0 / 6	0 / 6		
	#3	1 / 6	0 / 6	0 / 6		
63.0g (45x1.4)	#1	0 / 6	0 / 6			
	#2	0 / 6	0 / 6			
	#3	0 / 6	0 / 6			
67.5g (45x1.5)	#1	0 / 6				
	#2	0 / 6				
	#3	0 / 6				

Table C. Relative humidity at process start vs. decontamination efficacy.

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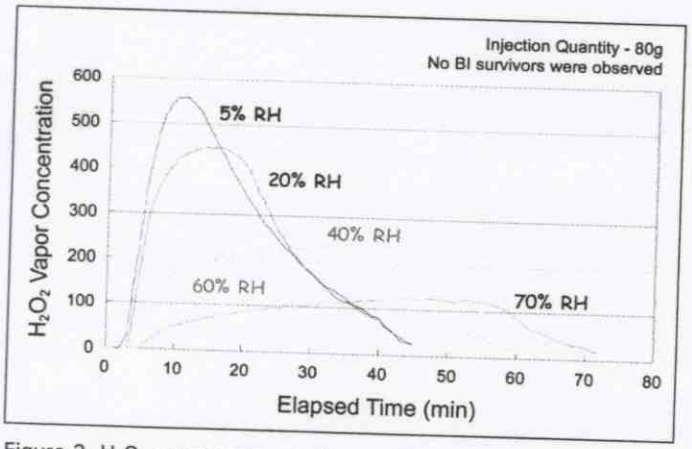

Figure 3. H_2O_2 vapor concentration vs. RH at start of cycle.

20g/minute resulted of injection times in this study of one to five minutes. The results given in Table A were extremely reproducible and are representative of multiple experiments. Figure 2 shows BIs placement for the isolator decontamination experiments.

Having established that effective VPHP decontamination could be achieved without continuous replenishment, a series of experiments were conducted to evaluate the effects of initial relative humidity on lethality. In these experiments, the RH was varied between 5% and 70% and the 80g total H_2O_2 injection quantity already demonstrated to be sufficient for complete inactivation of BIs was utilized throughout. The results given in Table B below show that effective kill was achieved from 5%-70% RH, these data indicate that relative humidity is a less critical factor in VPHP decontamination than had been previously thought.

Experiments were designed to examine the influence of humidity on VPHP efficacy and to evaluate RH levels throughout test cycles. A concentration monitoring system was used to determine the influence of humidity on the measured concentration of H_2O_2 vapor. Comparative experiments were done at relatively high and low humidity values. In this series of studies, a Draeger Polytron II monitoring system was used in the isolator enclosure. The results are presented in Figure 3.

The results of this experiment show that there does not appear to be a correlation between measured H_2O_2 concentration and the absolute microbiological efficacy of the process. An identical 80g total injection mass was used in each case, and complete inactivation of *Geobacillus stearothermophilus* spores on stainless steel coupons at a population of $>10^6$ were observed under all test conditions.

An additional study regarding the effects of humidity was conducted and the results are shown in Table C. The results of this study demonstrate that lower spore inactivation effectiveness is observed when relative humidity is low. It can be seen from these data that dehumidification prior to the start of injection is unnecessary and can be eliminated in most cases. Typically, cleanrooms and isolators operate at 40-60% RH which is suitable for VPHP decontamination. Since dehumidification is not required, the overall VPHP cycle or process time can be reduced.

The authors observed a new phenomenon relating to inactivation efficacy, condensation, aeration, and relative humidity. Data demonstrating this phenomenon is presented in Table D. During these test cycles, condensation was observed shortly after the start of the injection phase in each test shown in Table D. In the case of relatively low initial humidity, condensate will disappear soon after the completion of injection. At higher initial humidity levels, condensation was observed until aeration began. However, once aeration started, all condensate disappeared quickly and completely. At the start of aeration, the measured vapor concentration increased very rapidly as a direct result of evaporation of H_2O_2 from surfaces within the isolator enclosure.

Note: the studies shown in Table C were conducted under the same operating conditions as those shown in Table B. However, slight differences can be seen with respect to efficacy. These variations are the result of different BI placement and number of BIs used.

Table B includes results from BI locations that were "worst case" in terms of the difficulty to inactivate. On the other hand, Table C reflects data from BI locations chosen for relatively easy access.

Table D shows that the effective hold period for inactivation is 15 minutes after completion of injection regardless of initial humidity. The authors took particular note of this phenomenon in the refinement of this VPHP process. Table D suggests that spore inactivation is complete, or very nearly so at the end of the hold period. The authors also found that within the isolator the quantity of condensate is likely to increase during the hold period. Condensation continued to be observed after the conclusion of the 15-minute hold period and in the absence of aeration; however, no further increase in condensate was witnessed. These results indicate that physical phase change from vapor to liquid H_2O_2 appears to correlate with highly efficient spore inactivation. However, when BIs were placed into the isolator at the end of the hold period and held for a further 15 minutes, no kill was observed at 5-40% RH and only limited kill at 60% RH.

Experiments were conducted to evaluate the effect of temperatures on decontamination efficacy, and the results of this study are shown in Table E. Generally, cleanrooms housing isolators or used for aseptic processing are kept at temperatures in the range of 20-24°C. Therefore, temperatures of 20°C and 25°C were chosen for the study. It is important to note that since the system described in this article injects H_2O_2 at a rather high rate and only during the

Time / RH (%)	Survivors 10 minutes into Hold Period	Survivors at completion of 15 minute Hold Period	Survivors among BIs placed at the end of the Hold Period
5	2/6	0/6	6/6
20	2/6	0/6	6/6
40	1/6	0/6	6/6
60	2/6	0/6	4/6

Table D. Survivors as a function of process time.

Continued on page 102.

Starting Temperature	70% RH	60% RH	40% RH	20% RH	5% RH
20°C	0 / 30	0 / 30	0 / 30	0 / 30	0 / 30
25°C	0 / 30	0 / 30	0 / 30	0 / 30	0 / 30
Positives/Total BIs after 7 day incubation					

Table E. Lethality data - starting temperature vs. RH in isolator.

initial minutes of the cycle there is comparatively little effect upon the ambient temperature of enclosures compared to units that inject vaporous H_2O_2 in an approximately 100°C air stream throughout the process.

The data indicate that at a fixed total injection mass of 80g over an initial RH range of 5-70% complete kill of 10^6 population *G. stearothermophilus* biological indicators was achieved at 20° and 25°C. Therefore, no difference in efficacy could be seen between these two initial temperatures. Precise temperature control is clearly not necessary and kill efficacy is observed at the normal temperature and humidity ranges one would expect to encounter in a typical cleanroom, or an isolator housed within a cleanroom. Studies also were performed on the reproducibility of concentration as measured by the Dreager Polytron II. These data for three test cycles conducted at an initial RH of 40% and an 80g injection mass are presented in Figure 4. The differences observed within the three test runs shown are small and appear to be within the normal variability one should expect considering the experimental system and the analytical method.

Room Decontamination

To evaluate the scalability of the process described in this communication, a study was conducted in a pharmaceutical cleanroom with a total volume of 75m³. A total of 33 studies were performed at total injection volumes of 100-480grams. It was determined that 200grams was sufficient to achieve a consistent and reproducible cycle with complete inactivation of 47 *Geobacillus stearothermophilus* BIs with a population of $>10^6$ on each coupon.

Once this injection rate was found suitable, four additional test runs were performed under identical conditions to confirm reproducibility. Distribution fans were placed within the room to ensure uniform vapor distribution and deposition. Cycle parameters and results are shown in Table F. (Note: BI results and aeration outcome were the same for all five tests at the stated parameters).

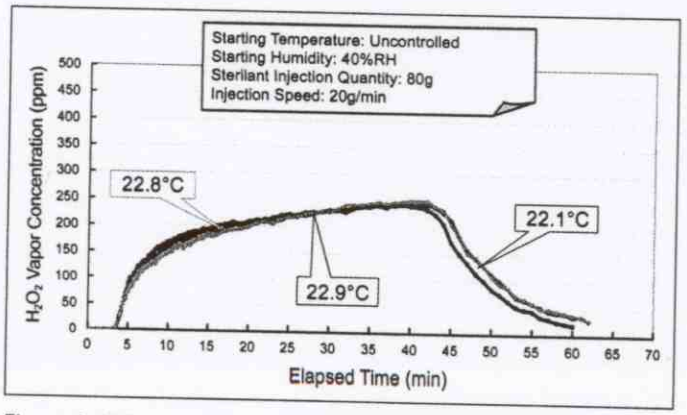

Figure 4. H_2O_2 vapor concentration reproducibility.

Discussion

There has been substantial debate on nearly all technical aspects of VPHP decontamination. This study was not designed to address the issues that have been the subject of debate, but rather to ask some fundamental questions relating to the practical aspects of hydrogen peroxide decontamination and to determine if a simpler, quicker, and more efficient method for VPHP could be developed. The data conclusively demonstrated that it is possible to develop a system that is highly efficacious and broadly applicable to a wide range of targets including isolators, and rooms.

The experiments found that VPHP is a robust process that is fully capable of decontamination at a wide variety of initial temperature and humidity conditions. Continuous or even semi-continuous replenishment of hydrogen peroxide during the process is unnecessary. It is clear that the VPHP process is capable of effective decontamination over a remarkably wide range of conditions. This is logical when one considers that H_2O_2 is one of the most powerful oxidants known. H_2O_2 has a higher oxidation potential than either Chlorine or Chlorine dioxide and the Hydroxyl radical which no doubt plays a vital role in H_2O_2 decontamination is exceeded in oxidation potential only by Fluorine. Therefore, the real challenge in VPHP decontamination or sterilization is not antimicrobial efficacy, but rather developing a process that is efficient as well as effective.

The studies also have demonstrated that the key factors involved in the development of an efficacious process are total injection volume and rapid injection of that total volume without replenishment. Thus, cycle development can be rather simple, because the hold time is fixed regardless of target volume, humidity, or temperature. These studies do indicate that although effective spore kill was seen over a range of 5-70%RH, the optimal range is 40-60% RH.

The discovery that continuous or semi-continuous injection is unnecessary and that initial dehumidification is not required can both simplify and shorten the VPHP decontamination process time. Furthermore, it must be remembered that anti-microbial processes are nearly always a trade off

Initial Temperature	20-24°C
Initial RH	50%
Total Injection Volume	200g
Injection rate	25g / minute
BIs placed	47 / test run
Total hold time	30 minutes
Total Process time including aeration	~ 5 hours
BIs Surviving	None
Residual at end of aeration	< 1ppm

Table F. 75m³ Cleanroom decontamination - parameters/results.

between kill effectiveness and damage to materials or product. Keeping the amount of H_2O_2 used to a minimum, allowing for some excess to ensure process robustness, is advantageous for avoiding unnecessary exposure of materials to this powerful oxidant and also realizing potentially shorter aeration times.

Also, we believe that insertion of BIs into the enclosure at the end of the 15-minute hold period did not result in efficient inactivation appears to indicate that a chemical change had occurred which resulted in greatly diminished oxidative capacity. Thus, it appears that the relationship of kill effectiveness to total injection volume most likely relates to providing sufficient VPHP to reach all surfaces within the enclosure or target. Longer hold periods and replenishment by further injection quantities do not enhance efficacy.

The information learned from these studies has served as a basis for the development of equipment designed around the processes described in this article. This system (patent pending) has been named HYDEC and has already been introduced commercially in Japan.

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