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Dry Hydrogen Peroxide for Viral Inactivation

Chris Lee and John R. Henneman

Abstract

Hydrogen peroxide is a common antiseptic and disinfectant that is effective against both enveloped and non-enveloped viruses, and it is sometimes used as a fumigant to achieve disinfection of indoor spaces. While it is effective as a fumigant, it cannot be used continuously, allowing for possible recontamination of the treated spaces between applications. A novel method of hydrogen peroxide application, termed “Dry Hydrogen Peroxide” (DHP™), generates molecules of hydrogen peroxide in a true gas state at concentrations low enough to be used continuously within spaces occupied by humans. This chapter explores the efficacy of DHP against a variety of viruses, both enveloped and non-enveloped. On surfaces, DHP achieved a $\geq 99.8\%$ reduction ($\geq 2.62 \log_{10}$ inactivation) of infectious H1N1 influenza A (enveloped) compared to the control condition within 1 hour, and it achieved a 99.8% reduction ($2.62 \log_{10}$ inactivation) of infectious feline calicivirus (non-enveloped) compared to the control condition within 6 hours. DHP also achieved a 99.8% reduction ($2.62 \log_{10}$ inactivation) of airborne MS2 bacteriophage (non-enveloped) within 1 hour in comparison to the control condition. These inactivation efficacy results, combined with results from recent clinical studies, indicate that DHP represents an effective adjunct technology that can mitigate viral load between intermittent applications of other types of disinfectants.

Keywords: viral inactivation, dry hydrogen peroxide, disinfectant, hydroxyl radical, biocidal action

1. Introduction

Since the late 19th century, hydrogen peroxide (H_2O_2) has been used as a disinfectant and antiseptic due to its potent antimicrobial properties against a wide range of pathogens [1]. Hydrogen peroxide attacks the essential external structures of pathogens (i.e. cell walls, viral envelopes, etc.) via a simple oxidation reaction, thereby weakening the pathogen's physical structure until it ultimately lyses from its own osmotic pressure [2–4]. Most commonly, H_2O_2 is used as a liquid antiseptic and disinfectant, but solutions of H_2O_2 are also vaporized and dispersed as a method of disinfection of indoor spaces. This process, however, requires the complete evacuation of personnel from the treated spaces, both during and for some time after the treatment, to protect human occupants from the toxic effects of the highly concentrated droplets [5, 6]. Symptoms of overexposure to H_2O_2 include irritation of the eyes, nose, throat, skin, and/or lungs, and concentrations over 75 parts per million (ppm) are considered “immediately dangerous to life or health” in humans [7, 8]. Droplets of vaporized hydrogen peroxide, depending on the generator, may contain concentrations

of approximately 400 ppm [9], therefore, while vaporized hydrogen peroxide is extremely effective as a sterilant, its potential for use in continuously occupied spaces is limited by its potency and potential toxicity to human occupants [10, 11].

Hydrogen peroxide is also an essential component of the human respiratory system, with human lungs maintaining an equilibrium concentration between 10^{-6} and 10^{-4} M via the lactoperoxidase system of enzymes [12]. Two enzymes within this system, known as the Duox compound, constantly produce hydrogen peroxide, while the third enzyme, lactoperoxidase, converts that hydrogen peroxide into an even stronger oxidizing agent, the hypothiocyanite ion (OSCN^-) [12, 13]. This enzymatic system allows the human body to tolerate low levels of hydrogen peroxide exposure without experiencing irritation or damage.

Recently, a new method of hydrogen peroxide generation and delivery termed Dry Hydrogen Peroxide (DHP™) was developed, with the goal of enabling safe continuous microbial inactivation to occur in occupied indoor spaces either when installed within an existing HVAC system or as a stand-alone device (**Figures 1 and 2**) [14]. DHP is produced by devices that include a 363 nm wavelength ultraviolet A (UV-A) bulb, which activates a proprietary photocatalyst that has been applied to a two-dimensional framed polyester mesh, referred to as a “sail”. Photons of UV-A radiation from the bulb excite electrons in the catalyst, promoting them to a higher energy state. This creates a positively charged “electron hole” in the valence band in the catalyst atoms, creating an active site. When ambient humidity (H_2O) is adsorbed into these active sites, an electron is scavenged from the water molecule. This causes a subsequent release of a proton (H^+) by the water molecule, and the resulting structure is a hydroxyl radical ($\text{OH}^\cdot$). The catalyst now has a free electron, a proton (H^+), and a hydroxyl radical available to perform oxidation reactions. Under normal circumstances, these three components simply combine to produce a water molecule in the gas phase. DHP technology, however, uses a proprietary plasma separation process to isolate hydroxyl radicals from the subatomic particles. This separation of the plasma allows for the hydroxyl radicals to combine and form stable molecules of hydrogen peroxide in a pure gas state (DHP), which are then dispersed throughout the space being treated. The subatomic particles that remain on the catalyst are then scavenged by ambient diatomic oxygen (O_2), forming more molecules of DHP by means of reduction. The concentrations of DHP that are produced through this process are well below the OSHA safety limit of 1 ppm, allowing the lactoperoxidase system to easily maintain the equilibrium concentration of hydrogen peroxide to the level naturally present in the lungs [12, 13]. Additionally,

Figure 1.
In-line Dry Hydrogen peroxide (DHP) device intended for use in an HVAC system.

Figure 2.
Stand-alone Dry Hydrogen Peroxide (DHP) device.

it has been confirmed that DHP devices produced by the patent holder do not produce ozone, according to Underwriter's Laboratories (UL) Standards 867 and 2998 [15, 16]. A recent study performed by Ramirez et al. reported no incidence of symptoms associated with hydrogen peroxide overexposure in pediatric oncology patients who were continuously exposed to DHP during their stay in a Pediatric Intensive Care Unit (PICU) [17].

Due to the novelty and mechanism of generation of DHP, this disinfection system is often confused with older technologies, such as vaporized hydrogen peroxide, bipolar ionization, and photocatalytic oxidation, though it is distinct from each of those technologies.

2. Dry hydrogen peroxide and vaporized hydrogen peroxide

While DHP and vaporized hydrogen peroxide both utilize hydrogen peroxide to reduce infectious pathogen burdens in a treated indoor space, there are several notable differences between the two technologies. The most apparent difference between DHP and vaporized hydrogen peroxide is that DHP is a true gas composed of individual molecules exhibiting near ideal gas behavior [18], whereas VHP is an aerosol of highly concentrated aqueous droplets. As a result, vaporized hydrogen peroxide effectively sterilizes a room, but it also may lead to aerosol H_2O_2 concentrations which exceed the safety limits for human exposure. Vaporized hydrogen peroxide may only be used in vacated areas. Other precautionary measures, such as sealing doors, windows, and HVAC systems, must be taken before use as well, in order to prevent unintended dissemination of H_2O_2 to adjacent spaces [5, 6, 9–11]. Further, in aqueous form, hydrogen peroxide forms a weak acid which is corrosive to some materials, equipment, and furnishings. Dry Hydrogen Peroxide, on the other hand, is much less concentrated, and does not cause such material compatibility issues. Dry Hydrogen Peroxide can be applied for an unlimited time of exposure and can be used in spaces occupied by humans. Dry Hydrogen Peroxide therefore represents a highly effective adjunct to the intermittent usage of harsher disinfectants.

2.1 Dry hydrogen peroxide and bipolar ionization

Bipolar ionization creates a plasma consisting of positive ions, negative ions, and free radicals, with the intention of releasing them into a space. This plasma can be generated in multiple ways, but the two primary types of bipolar ionization are

corona discharge and needlepoint. Both types of bipolar ionization utilize sets of oppositely charged electrodes to ionize ambient humidity and oxygen as the indoor air passes through the device. Corona discharge bipolar ionization is rarely utilized currently, due to the potential for generation of ozone; accordingly, most manufacturers have switched to needlepoint ionization [19]. Manufacturers of needlepoint bipolar ionization (NPBI) claim that the electrodes used in the devices produce an electric field with a voltage below 12 eV to eliminate the potential for ozone generation [20]. Dry Hydrogen Peroxide and bipolar ionization each utilize ambient humidity and oxygen in their generation processes and continuously disperse their products throughout treated spaces; however, DHP is produced as stable H_2O_2 molecules, while bipolar ions are an unstable plasma. Additionally, neutrally charged H_2O_2 generated from DHP can travel long distances, whereas the oppositely charged ions created by bipolar ionization may rapidly recombine, diminishing the effective concentration as distance from the device increases [21, 22].

2.2 Dry hydrogen peroxide and photocatalytic oxidation

Both DHP and Photocatalytic Oxidation (PCO) technologies utilize photocatalysis during their respective processes, however DHP devices are not PCO devices [18, 23]. DHP technology uses a plasma-separation process to specifically produce free H_2O_2 . Photocatalytic Oxidation technology, however, rapidly consumes any H_2O_2 that may form in the plasma, because H_2O_2 has a highly positive reduction potential (0.71 eV) and will be immediately reduced to water by subatomic particles in the plasma [24]. Photocatalytic Oxidation devices rely on a dense internal plasma zone within the device, but the microbicidal properties of the plasma only affect airborne microbes that circulate through the device, unless the device also produces ozone, which would impact microbes outside of the device.

2.3 Efficacy of DHP for inactivating viruses

Hydrogen peroxide's biocidal action against viruses relies on the oxidation of essential biomolecules that compose the external structures of the virus (i.e. lipid envelope, protein capsid, etc.) [2–4]. Both enveloped and non-enveloped viruses are susceptible to this mechanism, even though non-enveloped viruses are decidedly less susceptible [25]. A recent study indicated that DHP effectively reduced infectious burden of the enveloped coronavirus SARS-CoV-2 on surfaces in a laboratory setting, achieving an estimated 98.7% ($1.94 \log_{10}$) reduction compared to the corresponding control condition after 120 minutes in a simulated room environment [26]. Dry Hydrogen Peroxide was also associated with significant surface reductions in bacteria in two separate studies conducted in active hospital patient rooms [17, 27]. While these studies address DHP's efficacy against bacteria and enveloped viruses on surfaces, there have not yet appeared in the literature peer-reviewed reports detailing the efficacy of DHP against non-enveloped viruses or airborne enveloped viruses. The following sections will detail three previously unpublished laboratory trials that investigated DHP's potential for inactivating airborne viruses or viruses dried on surfaces.

2.4 Efficacy of DHP for inactivating influenza A H1N1

H1N1 is a strain of influenza A (family *Orthomyxoviridae*) that was responsible for a 2009 pandemic declared by the World Health Organization (WHO). Like SARS-CoV-2, H1N1 is an enveloped virus, and it has been known to remain infectious on non-porous surfaces, such as glass and stainless steel, for 24–48 hours

[28, 29]. A DHP device was tested against titers of H1N1, with a starting TCID₅₀ of 6.05 log₁₀, in a laboratory biosafety hood to determine if DHP effectively inactivated the virus in comparison to the control condition after 120 minutes exposure (**Tables 1** and **2**).

Aliquots of diluted stock H1N1 were used to inoculate 1" × 1" squares on the center of 1" × 3" glass slides that had previously been sterilized and autoclaved. The slides were then placed into plastic Petri dishes. Ten slides, in total, were prepared in this way, with duplicates for each timepoint: Time Zero, T = 60 minutes Virus Control, T = 120 minutes Virus Control, T = 60 minutes Virus Test Carrier, T = 120 minutes Virus Test Carrier. Once inoculated with virus, the slides were allowed to dry for 25 minutes at 24°C and 36% relative humidity. The dried carriers were placed in their respective laboratory hoods, one of which was currently being treated with a DHP device that had been operating for 12 hours to precondition the space. The Time Zero samples were immediately collected and eluted with 2 mL of Influenza Infection Medium (EMEM supplemented with 0.125% w/v bovine serum albumin + 1 µg/mL TPCK-trypsin + antibiotics). Serial dilutions were then performed to the 10⁻⁵ dilution and plated in quadruplicate onto MDCK (dog kidney) monolayers. At the designated timepoints, the T = 60 and the T = 120 samples were harvested and enumerated in an identical fashion to the Time Zero samples. The assay trays were then incubated at 35°C on an orbital rotator (60 rotations/minute) for 60 minutes. Once the virus-host cell adsorption had completed, the trays were removed from incubation, and 1.0 mL of the Influenza Infection Medium was pipetted into each well of the assay plate for each of the samples. The MDBK wells were then incubated for 7 days. All titers were determined using the Spearman-Kärber method [30].

After the incubation was complete, the wells were scored for viral cytopathic effect (CPE), and the Tissue Culture Infectivity Dose at the 50% Endpoint Dilution (TCID₅₀) was calculated for each pair of samples (**Table 2**). In comparison to the control, the DHP-treated samples yielded a ≥ 2.62 log₁₀ reduction in virus titer at 60 minutes and a ≥ 1.87 log₁₀ reduction at 120 minutes. The log₁₀ reduction in titer observed at 60 minutes corresponds to a percent reduction of ≥99.8%, compared to the control condition (**Table 2**) [31].

Virus	Strain	Cell line	Description	Culture medium
Influenza A (H1N1) ^b	A/PR/8/34	MDCK	Canine Kidney	EMEM + 0.125% bovine serum albumin w/v + 1 µg/mL TPCK-trypsin + antibiotics
Feline Calicivirus ^c	ATCC VR-782	CRFK	Feline Kidney	MEM + heat-inactivated fetal bovine serum +100 units/mL penicillin +10 µg/mL gentamicin +2.5 µg/mL amphotericin B
MS2 Bacteriophage ^d	15597-B1	<i>E. coli</i> 15597	Gram Negative Bacteria	50% Tryptic Soy Agar

^aAbbreviations used: ATCC, American Type Culture Collection; CRFK, Crandel-Reese Feline Kidney; EMEM, Eagle's Minimum Essential Media; MDCK, Madin-Darby Canine Kidney; MEM, Minimum Essential Media.
^bTesting performed at Antimicrobial Test Laboratories, Round Rock, Texas, USA.
^cTesting performed at ATS Labs, Eagan, MN, USA.
^dTesting performed at Microchem Laboratory, Round Rock, TX, USA.

Table 1.
Summary of viruses and detector cells used in these efficacy studies^a.

	Influenza virus titer (TCID ₅₀ /mL)		
	Time zero	T = 60 min	T = 120 min
Control	6.05	4.80	3.80
DHP-Treated	6.05	≤2.18	≤1.93
Log ₁₀ Inactivation*		≥2.62	≥1.87
Percent reduction*		≥99.8%	≥98.6%

*Compared to Control.

Table 2.
Inactivation of influenza virus H1N1 over time by exposure to Dry Hydrogen Peroxide (DHP).

2.5 Efficacy of DHP for inactivating feline calicivirus

Feline calicivirus (FeCV) is a non-enveloped, single-stranded RNA virus (family *Caliciviridae*) that is often used as a surrogate in laboratory testing to simulate human norovirus, a major cause of gastrointestinal hospital-acquired infections (HAIs) [32, 33]. On non-porous surfaces, FeCV has been found to remain viable for 12–72 hours [34]. The efficacy of a prototype DHP device was tested against titers of FeCV, with a starting titer of 6.6 log₁₀ TCID₅₀/mL, over the course of 24 hours (Tables 1 and 3).

Aliquots of FeCV (ATCC VR-782) were inoculated onto glass slides with an accompanying organic soil load of ≤1% fetal bovine serum (FBS) to simulate contamination in a physiological matrix. The original titer of the input virus control was approximately 8.0 log₁₀/mL, but after being allowed to dry on the carriers, the FeCV titer had decreased to an average of 6.6 log₁₀/mL. For both the control and treatment groups, duplicate samples were collected at each timepoint (Time zero, T = 2 hours, T = 6 hours, T = 24 hours). After drying of the virus onto the slides was complete, the carriers were placed in their respective biosafety laboratory hoods, and the DHP device was activated in the hood containing the treatment group of samples. Temperature and humidity levels remained between 21 and 24°C and 36–39%, respectively, throughout the duration of the experiment. The test carriers were retrieved and scraped to resuspend the contents at the designated timepoints. Each sample’s contents were transferred to a sterile tube and then serially diluted in the test medium (MEM supplemented with inactivated FBS, 100 units/mL penicillin, gentamicin, and 2.5 µg/mL amphotericin B). Once diluted, a cell-based infectivity assay involving Crandel Reese feline kidney (CRFK) cells was used to determine infectious titer.

The average titer (TCID₅₀/mL) for each pair of samples was then calculated (Table 3). DHP-treatment resulted in FeCV inactivation (1.5 log₁₀ after 2 hours,

	Feline calicivirus titer (TCID ₅₀ /mL)			
	Time zero	T = 2 hr	T = 6 hr	T = 24 hr
Control	6.6	5.8	5.1	3.4
DHP-Treated	6.6	4.3	2.3	≤0.6
Log ₁₀ Inactivation*		1.5	2.8	≥2.8
Percent reduction*		96.8%	99.8%	≥99.8%

*Compared to Control.

Table 3.
Inactivation of feline calicivirus over time by exposure to Dry Hydrogen Peroxide (DHP).

and 2.8 log₁₀ reduction after 6 hours of exposure time). The 2-hour and 6-hour log₁₀ reductions in infectious titer correspond to 96.8% and 99.8% inactivation, respectively, in comparison to the control condition (Table 3) [35].

2.6 Efficacy of DHP for inactivating MS2 bacteriophage

MS2 is a single-stranded non-enveloped RNA bacteriophage that often infects *Escherichia coli* (*E. coli*), and has been used as a surrogate for human norovirus and other non-enveloped viruses. MS2 bacteriophage has been shown to survive on non-porous surfaces for 4–10 days, which is aligned with the length of time norovirus can survive under similar conditions [36, 37]. The efficacy of a DHP device against airborne MS2 bacteriophage was investigated over the course of 4 hours (Tables 1 and 4).

This trial was conducted in an aerobiology chamber with a volume of ~30 m³ to simulate the conditions of the DHP device’s intended use more accurately. The test inoculum containing a titer (~5.0 log₁₀/mL) of MS2 bacteriophage strain 15597-B1 was split equally and added to two separate nebulizers within the test chamber. These nebulizers were then activated inside the chamber for 60 minutes before the Time Zero sample collection occurred, using an SKC bio-sampler (500 L) equipped with phosphate buffered saline. The sample was then serially diluted and plated in 50% Tryptic Soy Agar (TSA) containing *E. coli* to facilitate the accurate enumeration of the remaining infectious MS2 bacteriophage. Subsequent samples were then collected each hour for the following four hours, with no DHP present, to serve as the no-treatment control. Once the chamber had been adequately decontaminated, the solutions containing the MS2 bacteriophage were again added to the nebulizers. The DHP device was activated after the collection of the Time Zero sample, and subsequent sample collections were performed identically to the control samples. All plated samples were then incubated for 24 hours, and the plaque-forming units (PFU) of MS2 were enumerated. A reduction in log₁₀ PFU relative to the untreated control condition is indicative of extent of inactivation.

The Time Zero samples yielded counts of 5.84 × 10⁴ and 5.83 × 10⁴ PFU for the control and DHP-treated groups, respectively. After an hour of exposure to DHP, the count of plaques formed by destroyed *E. coli* decreased by 3.54 log₁₀ to 1.70 × 10¹ PFU, whereas the corresponding untreated control sample decreased by 0.83 log₁₀ to 8.61 × 10³ PFU. Compared to the untreated control, DHP achieved a 2.71 log₁₀ reduction in infectious airborne MS2 bacteriophage titer after 1 hour of exposure, which corresponds to a 99.8% reduction (Table 4) [38].

	MS2 bacteriophage titer (<i>E. coli</i> PFU/mL)				
	Time zero	T = 1 hr	T = 2 hr	T = 3 hr	T = 4 hrs
Control	5.84 × 10 ⁴	8.61 × 10 ³	2.20 × 10 ³	5.83 × 10 ²	7.59 × 10 ²
DHP-Treated	5.83 × 10 ⁴	1.70 × 10 ¹	≤1.68 × 10 ¹	≤1.58 × 10 ¹	≤1.62 × 10 ¹
Log ₁₀ Inactivation*		2.70	≥2.12	≥1.57	≥1.67
Percent Reduction*		99.8%	≥99.2%	≥97.3%	≥97.9%

*Compared to control.

Table 4.
Plaque-forming units (PFU)/mL for *E. coli* infected with MS2 bacteriophage over time after exposure to Dry Hydrogen Peroxide (DHP).

3. Discussion and conclusions

United States Food and Drug Administration guidance [39] and the literature [40] suggest that small non-enveloped viruses are generally less susceptible to inactivation of germicidal chemicals, such as hydrogen peroxide, than enveloped viruses, vegetative bacteria, and vegetative fungi. The virucidal efficacies displayed in these three surface and air inactivation studies indicate that DHP is capable of reducing surface and air concentrations of both enveloped and non-enveloped viruses. Therefore, it can be reasonably expected that DHP will be capable of similar microbial efficacy against vegetative bacteria and fungi as well, a hypothesis that is strongly supported by microbial reductions observed in the presence of DHP in healthcare settings [17, 27].

Within healthcare settings, the environmental microbial load is strongly associated with the risk of developing an HAI, and effective reduction of environmental microbial load has been shown to greatly mitigate that risk [41, 42]. It might seem prudent to rely on the most powerful, broad-spectrum disinfectants, such as full-strength VHP, caustics, or chlorine dioxide fogging, which are capable of inactivating pathogens to levels that approach sterile conditions. Those types of disinfectants, unfortunately, can only be applied intermittently. Reliance on intermittent methods of disinfection has repeatedly failed to demonstrate a consistent and effective reduction in environmental bioburden [43]. It is apparent that, for strong disinfectants to achieve their full potential, these must be accompanied by an adjunct method of continuous microbial reduction that can mitigate levels of bioburden during the intervals between the periodic application of the other disinfectants.

In the wake of the SARS-CoV-2 pandemic which caused the COVID-19 disease, there is a unique and universal awareness of the need for effective surface and air hygiene methods in the commercial, educational, and residential sectors. This increased demand for technologies that successfully mitigate environmental pathogen load in sectors outside of healthcare further stresses the need for simple, accessible, and automated adjunct technologies to accompany intermittent microbial application protocols and disinfectant usage. The repeated demonstration of the efficacy of DHP against a variety of pathogens in laboratory and field settings, its lack of human toxicity at the H_2O_2 concentrations used, and the material compatibility associated with DHP and its breakdown products (O_2 and H_2O) qualify the technology as a strong contender for meeting this demand.

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