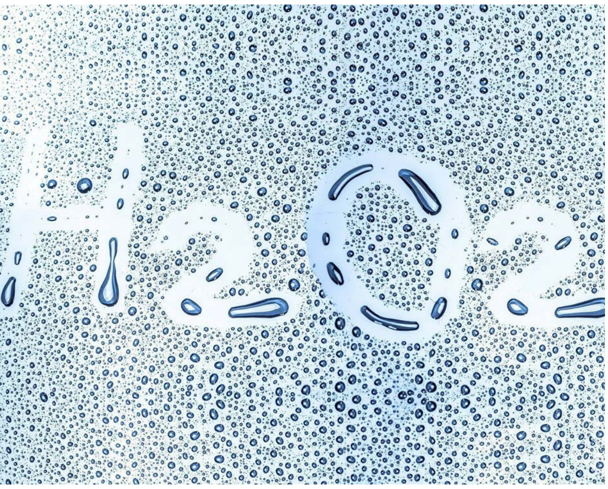


Hydrogen Peroxide Decontamination: Vapour and Aerosol Comparison

Hydrogen peroxide (H_2O_2) disinfection systems for automated decontamination of environments have received increased interest due to the Sars-CoV-2 pandemic and the growing importance of sanitization. Bio-decontamination with hydrogen peroxide is a low-temperature method to reduce the bio-burden and represents an alternative to thermal disinfection, which is not always applicable, especially when heat sensitive equipment must be processed. Hydrogen peroxide is a good choice as chemical agent to achieve low-temperature bio-decontamination goals.

Despite decontamination with hydrogen peroxide is nowadays widely used in many fields of industry and research (including healthcare, pharmaceuticals, food and beverage, bio-safety laboratories, and animal facilities), the chemical and physical mechanism behind the efficacy of this substance against a wide range of microorganism is still mystical and not well understood. This white paper focuses on automatic airborne disinfection systems (AADSs) that use hydrogen peroxide as chemical agent, in vapour or aerosol form, and how these two methods act to achieve a rapid sporicidal effect. Moreover, this white paper examines the differences between the two technologies and their efficacy. Before doing a comparison between vapour phase hydrogen peroxide (VPHP) and aerosolized hydrogen peroxide (aHP), and try to figure out which method is more suitable for a particular application, it is necessary to understand better this chemical agent and why it is a rapid sporicidal at low temperature. The term "micro-condensed hydrogen peroxide" (MCHP) will be introduced as an aid to understanding how the process truly acts and this understanding helps to generate robust sanitisation cycles[24]. This new knowledge can be applied to the many devices becoming commercially available for environment (as enclosures, isolators, pass-through chambers, labs, rooms) bio-decontamination.

1. SOME USEFUL DEFINITIONS

Decontamination (or Decon) is the process of cleansing an object or substance to remove contaminants such as micro-organism or hazardous material, including chemicals, radioactive substances and infectious disease[1].

Disinfection is defined as the destruction of micro-organism, except bacterial spores, on inanimate objects (if deal with living tissue is antisepsis). Three level of disinfection are achievable depending on the amount and kind of microbial killing involved[2].

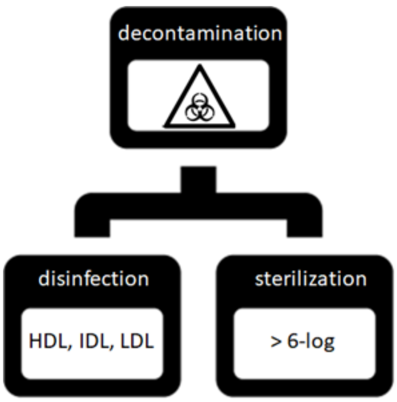
These levels of disinfection are as follows:

- High Disinfection Level (HDL): the destruction of all viruses, vegetative bacteria, fungi, mycobacteria, and some, but not all, bacteria spores[3].
- Intermediate Disinfection Level (IDL): the destruction of all mycobacteria, vegetative bacteria, fungal spores, and some nonlipid viruses, but not bacterial spores.
- Low Disinfection Level (LDL): a process that can kill most bacteria (except mycobacteria or bacterial spores), most viruses (except some nonlipid viruses), and some fungi.

Sterilization refers to any process that remove, kills, or deactivate all forms of life (viruses, bacteria, fungi, spores, etc…) and other biological agents, like prions, present in a specific surface, object, or fluid, for example food or biological culture media[4].

Disinfection and Sterilization are form of Decontamination as shown in Figure 1.

Cleaning is the process of removing residues and soil (dirt, dust, protein, skin cells and so on) from surfaces to the extent they are visually clean. Cleaning ensures that a disinfectant will work effectively and interact with the microbial cells. A detergent is a chemical that penetrates soiling and reduces the surface tension (which fix the soil to the surface) to allows its removal. Cleaning can remove or dilute microbial population and many detergents contain chemical additives that can "disinfect", but a detergent is not a disinfectant.



Log Reduction is a measure of how thoroughly a decontamination process reduces the concentration of a contaminant. A "log reduction" (technically a reduction by one order of magnitude) represents a 90% reduction in microbial population. As a "rule of thumb" the sterilization target is usually at least a 6-log reduction of micro-organism (spore) population.

6-log reduction → 99.9999% reduction of the microbial population

D-Value (or decimal value) is the time required, at a given condition or set of conditions, to achieve a log reduction, that is, to kill 90% (or 1 log) of relevant micro-organisms. Higher D-Value means higher resistance of the microbial strain to the decontamination process. The D-value is a function of sterilization conditions and varies with the type of micro-organism, temperature, water activity, pH, etc.

1.2 BIO-DECONTAMINATION

A 6-log reduction is a common goal of bio-decontamination, whereas the goal of "sterilization" is to kill all microorganisms, viruses, and spores. Bio-decontamination is used due to its relative safety for operators, equipment, and materials and the bio-decontaminated status can be achieved through different methods:

- **Heat:** Steam (autoclave), Dry Steam.
- **Chemical Sterilization:** Ethylene Oxide (EtO), Hydrogen Peroxide (H_2O_2), Formaldehyde, Chlorine Dioxide (ClO_2).
- **Radiation:** Ionizing Radiation (γ-ray), non-ionizing radiation (UV).
- **Filtration.**

2. HYDROGEN PEROXIDE

Hydrogen peroxide is a chemical compound, with formula H_2O_2, known also as "oxygenated water" and discovered in 1818 by the French chemist Louis Jacques Thenard. In its pure form, it is a liquid, colourless solution slightly more viscous than water, with a boiling point of $\simeq$150°C. It is always used as aqueous solution at different volumetric concentration (see table 1).

H_2O_2 concentration	Density	Volume*
3%	≃ 1 g/ml	10
30%	1.100 g/ml	111
35%	1.131 g/ml	133
40%	1.200 g/ml	154

Table 1

** Volume: liters of oxygen released by 1 liter of H_2O_2 solution (in standard condition, 0°C and 1 bar).*

Hydrogen peroxide is an unstable compound which naturally decomposes to form water and oxygen according to the exothermic reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2 + \textbf{196.2 kJ}$$

The rate of decomposition increases with rise in temperature, concentration, light, and pH. Decomposition is catalysed by various elements, including metal and their compounds (manganese dioxide, silver, and platinum)[6].
Hydrogen peroxide can also be decomposed biologically by the enzyme catalase. Microorganisms having such enzyme, show an increased resistance to hydrogen peroxide oxidation (e.g. staphylococcus aureus).
Hydron peroxide can be used as an oxidizer, bleaching agent, propellant in rocketry and antiseptic. Concentrated hydrogen peroxide is a reactive oxygen spices (ROS) and causes cell's membrane proteins, lipids, and DNA denaturation through oxidation processes.
In the late 1980s, American Sterilizer Company (now STERIS) discovered that hydrogen peroxide in vapour phase rapidly kills spores.

3. HYDROGEN PEROXIDE EFFICACY

Hydrogen peroxide has been shown to be efficient against a wide range of microorganism including vegetative bacteria, viruses, mycobacteria, fungi, yeasts, and spores (Table 3). The mechanism of action is related to the oxidizing power (see table 2) of hydrogen peroxide, according to the following reaction.

$$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H_2O$$

Veget. Bacteria	Viruses	Fungi & Yeasts	Bacterial Spores
Staphylococcus aureus[10]	*Avian Influenza Virus (AIV)*[14]	*Candida Albicans*[18]	*Bacillus Anthracis*[13]
Escherichia Coli[9 10]	*Human Norovirus*[16]	*Candida Shehatae*[17]	*Bacillus Subtilus*[13]
Acinetobacter Baumanii[11]	*Coronavirus*[14, 15]	*Candida Succiphila*[17]	*Geobacillus Stearothermophilus*[7, 11, 13]
Klebsiella pneumoniae[9]	*Rotavirus*[16]	*Scheffersomyces Stipitis*[17]	*Clostridium Botulinum*[12]
Pseudomonas aeruginosa[10]	*Adenovirus*[14, 16]	*Mycobacterium Tubercolosis*[20]	*Clostridium Difficile*[8, 9, 11]
Enterococcus Hirae[10]	*Influenza A (H1N1)*[15, 16]	*Aspergillus*[19]	*Bacillus atrophaeus*[21]

Table 2

Besides, hydrogen peroxide can be used against prions, which are infective misfolded proteins, hard to be inactivated, responsible for Transmissible Spongiform Encephalopathies (TSE)[23].
Hydrogen peroxide has been shown to be effective against multi drug resistant bacteria, which are responsible for nosocomial infections in hospitals as: clostridium difficile associated disease, vancomycin-resistant enterococcus (VRE) and methicillin-resistant staphylococcus aureus (MRSA)[22] are all associated with environmental contamination.

Oxidant	Oxidation Potential, V
Fluorine	3.0
Hydroxyl radical	2.8
Ozone	2.1
Hydrogen peroxide	**1.8**
Potassium permanganate	1.7
Chlorine dioxide	1.5
Chlorine	1.4

Table 3

Finally, the efficacy of hydrogen peroxide against the coronavirus family, including the virus responsible of the current pandemic, Sars-Cov-2 (Covid-19), has been shown in many studies[14, 15].

4. AUTOMATIC AIRBORNE DISINFECTION SYSTEM - AADS

An automatic airborne disinfection system (AADS) is a device able to generate a mixture of air and disinfectant agent, which spreads into the environment to be decontaminated touching all the surfaces exposed. Such kind of devices are designed to automatically decontaminate environments, such as rooms, pass-through chambers, small pass-boxes, or isolators, etc… as well all the miscellaneous equipment, tools and materials contained into the environment. In this article we focus on AADSs that use hydrogen peroxide as chemical disinfectant agent, nevertheless there are alternative agents that can be used to achieve the bio-decontamination. The choice of the right decontaminant agent is related to the chemical compound efficacy, compatibility with materials, toxicity, presence of toxic by-products, and cost.

5. ALTERNATIVE CHEMICAL AGENTS

- **Formaldehyde:** nowadays is almost out of use because of its carcinogenicity and toxicity.
- **Ethylene Oxide:** is widely used for medical device due to its ability to penetrate hard-to-reach area, but it needs classified environment due to its explosivity (ATEX compliance) and extensive aeration due to its carcinogenicity.
- **Chlorine Dioxide:** is a natural gas with antimicrobial activity, obtained through oxidation. It extremely corrosive on stainless in presence of water.

On the other hand, **Hydrogen Peroxide:**

- is a great alternative to other toxic compound and it has non-toxic by-product (just water and oxygen).
- Is effective at low temperature, so indicated for temperature sensitive materials.
- has an excellent material compatibility.
- does not need lengthy aeration.
- is a repeatable process, easy to be validated.
- can penetrate HEPA filters.

6. TWO MAIN DELIVERY METHODS (VAPOUR AND AEROSOL)

There are to different types of hydrogen peroxide based automatic airborne disinfection systems (AADSs) available. Some are based on **hydrogen peroxide vapour (Vapour Phase Hydrogen Peroxide or VPHP)** and others are based on **aerosolised hydrogen peroxide (aHP).**

The main difference between the two methods is the physical state of the hydrogen peroxide escaping from the decontamination system. The VPHP system vaporises 30÷35 % hydrogen peroxide aqueous solution to produce a mixture of air and hydrogen peroxide vapour, which can be considered with good approximation, a gaseous system, where the single molecules are not bound each other. On the other hand, aHP system produces a mist (also known as fog) by aerosolising a solution containing a lower concentration of hydrogen peroxide solution (typically lower than 8%) and other chemical additives (typically silver ions, peracetic acid, corrosion inhibitors, etc…).

Fig.1: Active Pass-through chamber

The resulting mist / fog comprises droplets ranging from 5÷20µm in diameter, which fluctuates throughout the environment thanks to their kinetic energy, still in a liquid state (fogging process).

If we compare the hydrogen peroxide molecule's dimension (order of 0.1nm) to the smallest feasible droplet (order of 10µm), we can observe a difference of five order of magnitude (1 to 100,000). It means that if the vapour were a grain of sand, the aerosol would be a hot-air balloon. For this reason, we can figure out from now that hydrogen peroxide in vapour phase has higher penetrating power and better distribution ability than aerosol.

VPHP systems typically use a stabilized aqueous solution of hydrogen peroxide 30÷35 % concentrated (volumetric concentration), while for aHP generator the aqueous solution concentration of hydrogen peroxide is ≤8%. In some case, the solutions used for AHP technology, have little percentage of additives (as Peracetic Acid, silver ions, anti-corrosive agent, iso-propylic acid, etc...), which enhance the killing power of the chemical agent or its material compatibility.

7. MICRO-CONDENSED HYDROGEN PEROXIDE

AADSs generate a mixture of hydrogen peroxide (vapour or aerosol) and air that spreads into the environment, with the scope of touching all the surfaces to be decontaminated. What does it happen then? Does the hydrogen peroxide, vapor or aerosol,"kill the bugs"? What is the mechanism that allows to achieve the rapid sporicidal effect?

7.1 VPHP process

To answer these questions, we firstly consider the decontamination with **hydrogen peroxide in vapour form (VPHP)**, which is also historically the first method discovered to be rapidly effective against spores. When hydrogen peroxide in vapour phase is injected into an environment, the gaseous concentration of H_2O_2 grows up. The concentration is normally expressed in volumetric part per million (ppm), which is a unitless parameter used to measure a small amount of chemical concentration. The figure 2 shows a typical theoretical hydrogen peroxide concentration trend, during a decontamination cycle with vapour phase hydrogen peroxide, in which it is possible recognize four phases:

- **Dehumidification:** during this phase, the air water vapour content is brought down. It is a facultative phase, but it is helpful to increase the amount of hydrogen peroxide injectable and to make the bio-decontamination cycle repeatable.
- **Rising:** during this phase, hydrogen peroxide is injected and vapourised at high rate to reach the target value of concentration (400 ÷ 1500 ppm) as soon as possible.
- **Plateau:** during this phase, the target concentration of hydrogen peroxide vapour is maintained constant until the 6-log reduction of microbial population is achieved. Normally the rate of injection of Hydrogen Peroxide is lower than during the Rising phase.
- **Aeration:** this is the wash-out phase, where the vapour of hydrogen peroxide is removed and the concentration level slow down. When the concentration is under the permissible exposure limit (PEL) of 1 ppm, the environment is again accessible.

The rapid sporicidal effect is achieved with very low concentration of hydrogen peroxide vapour (hundreds of ppm). Theoretically, such level of concentration should take many hours of contact to get 6-log reduction in microbial population, instead the time experimentally observed was in the order of minutes.

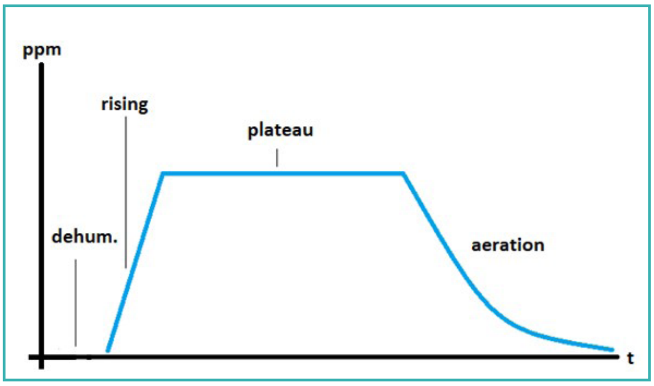


Fig.2: Theoretical trend of hydrogen peroxide ppm concentration during a typical bio-decontamination cycle

Significant research on the VPHP process was made by Watling and Parks (2004)[25]. If the concentration of hydrogen peroxide vapour is measured throughout a classic four-phase cycle, something strange happens when the plateau phase ends and the aeration phase starts. At this point, the supply of vapour is cut off and the concentration of the vapour should immediately begin to fall away. Paradoxically it does not. Instead, it increases quite significantly for a short time before to start to decrease quickly (figure 2). The work of Watling and Parks (2004)[25] offered a plausible explanation. Vapour Phase Hydrogen Peroxide condenses onto the surfaces in a manner not visible to the naked eye. The condensation is in the form of micro-droplets (few microns) with a high concentration of hydrogen peroxide, perhaps 60% or 70%.

It is the presence of this liquid hydrogen peroxide solution, at high concentration, on the surfaces that produces the very rapid kill observed. This phenomenon is called Micro-Condensed Hydrogen Peroxide (MCHP) and it is responsible for the very rapid sporicidal effect (6-log reduction achievable in minutes)[24]. Indeed, the airborne disinfectant mixture, composed by water and H_2O_2 vapours, condense. MCHP happens because the hydrogen peroxide has a higher molecular weight than water, therefore H_2O_2 has a much lower vapour pressure than water. It means that Hydrogen Peroxide condenses out preferentially ahead of water vapour, which is inevitably present when an aqueous solution is used as the source.

Functional principle of VPHP process

Many suppliers of VPHP system (the two major players are Steris and Bioquell) are present in the market in a quite competitive scenario. All generators present in the market differ each other for some features, normally these differences are more commercial than technical. The functional principle of all the VPHP generator is always the same. A ventilator generates a controllable airflow (the carrier) that passes through a vaporizer (normally a metal hot plate), where the solution of hydrogen peroxide is injected at controlled rate (e.g. through a peristaltic pump) and instantaneously vapourised (flash vaporisation). The airflow captures the hydrogen peroxide vapour and is routed into the environment to be decontaminated (see Figure 3). The VPHP generator can be connected to environment to be decontaminated in open loop or closed loop modality. The latest can be further divided in closed loop single pass or closed loop double pass.

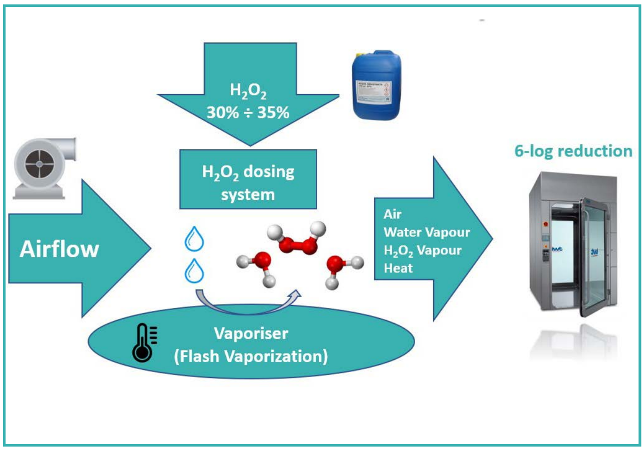


Fig.3: VPHP generator: generic functional scheme.

The open loop modality foresees that the vapour of hydrogen peroxide exhausts from the environment, through dedicated ducts (dumpers and fans can also be present), see figure 4. The closed loop modality foresees that the hydrogen peroxide vapour returns to generator. In case of closed loop single pass configuration, the hydrogen peroxide vapour is catalysed, dehumidified and re-injected (see figure 5). In case of closed loop double pass configuration, the hydrogen peroxide vapour is recirculated through the environment to be decontaminated, injecting just small dose to compensate the degradation, until the plateau phase ends.

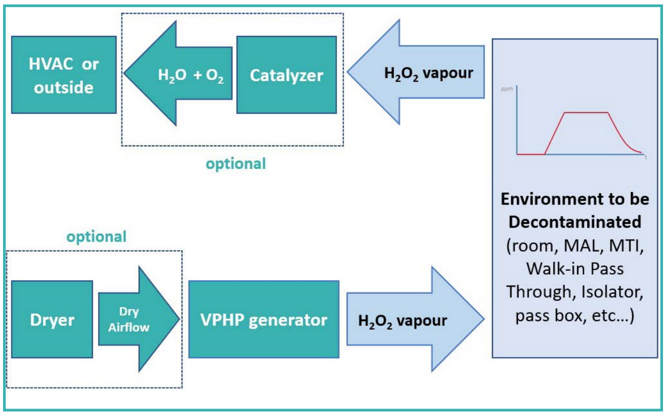


Fig.4: VPHP generator in open loop modality.

Then the injection stops, and the air is recirculated through a catalysing unit to perform the aeration phase. A valves system allows the switching between the two loops of this configuration, see figure 6. The choice between the different configuration must consider if the chamber, the enclosure or the room has or not its own exhaust system, able to accept hydrogen peroxide vapour (as HVAC or dedicated catalysing unit). If there is no possibility to exhaust vapour of hydrogen peroxide the closed loop configuration must be adopted.

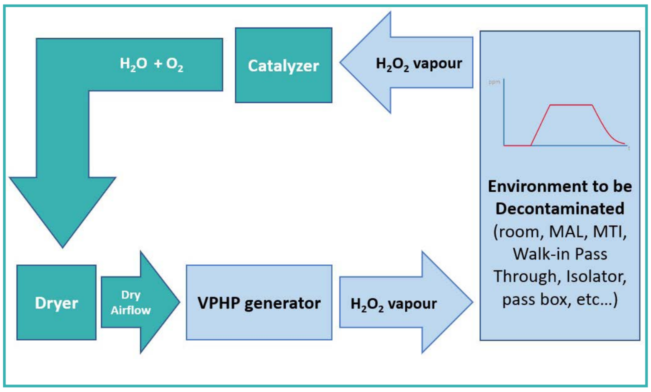


Fig.5: VPHP generator: closed loop single pass.

The open loop modality has capability for higher concentration of hydrogen peroxide and the presence of an external exhausting system usually makes the aeration phase shorter. On the other hand, the closed loop modality has lower hydrogen peroxide consumption, and it does not need any external ventilation system for work.

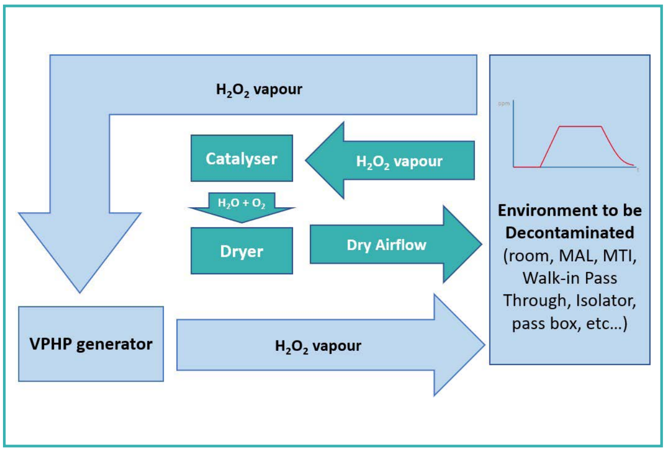


Fig.6: VPHP generator: closed loop double pass.

7.2 Aerosolized Hydrogen Peroxide process

In contrast, aHP systems produce a mechanically generated aerosol (e.g. by pressure). The bio-decontamination cycle with aerosolized hydrogen peroxide can be divided in three main phases:

- **Nebulization:** during this phase, the hydrogen peroxide solution (concentration ≤ 8%) is aerosolized. The total amount of solution transformed in fine mist depends on the volume to be treated (g/m^3 or ml/m^3).
- **Contact time:** during this phase, the system stops to produce aerosol and the micro-droplets created gets in contact with all the reachable surfaces. The contact time must be tuned in order to get the target log reduction through the action of MCHP.
- **Aeration:** during this phase, the hydrogen peroxide must be removed from the environment. The removal can be achieved in two ways: by using an external catalysing recirculating unit (active aeration) or by waiting for hydrogen peroxide degradation (passive aeration). In any case the level of hydrogen peroxide at the end of this phase must be lower than 1 ppm (PEL).

Nevertheless, Aerosolized Hydrogen Peroxide has a very rapid sporicidal effect too. Since the aerosol has a huge exchange surface, the evaporation of the small droplets is very fast (evaporation velocity is inversely proportional to the drop size). The most likely hypothesis is that the aerosolized hydrogen peroxide, which has a huge exchange surface, evaporates very quickly and then develops micro-condensed hydrogen peroxide onto surfaces.

Functional principle of aerosolised Hydrogen Peroxide (aHP)

If the producers of VPHP generator are a lot, the producer of aHP generator are even more. The last pandemic period has seen the appearance of a significant number of new suppliers. Aerosol systems produce mist by nebulizing a solution containing low concentration of hydrogen peroxide (< 8%) and other chemicals. The aerosol is generated by pressure applied upstream a calibrated nozzle (see figure 7). Nowadays the modern technologies allow to control with good precision the droplet dimension, that ranges between 5÷20µm. In some case, as Steramist® by TOMI or Zherox® by AMInstruments, the aerosol generated pass through an electrical field with an high potential difference (17000 V). This technology is called ionized hydrogen peroxide (iHP). It is hypothesised that the droplets retain a positive charge enhancing their distribution and generating more oxygen reactive species.

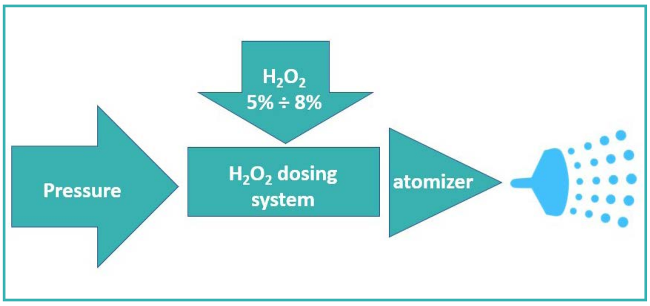


Fig.7: Aerosolised Hydrogen Peroxide generator

All the producer of aHP or iHP have their own proprietary solution of hydrogen peroxide, and all have a concentration lower than 8%. The proprietary recipes contain in some case additives to boost the killing power of hydrogen peroxide, as silver ions (HaloMist® by Halosil or Nocolyse® by OxyPharm), or peracetic acid (O2Safe® by Phileas). In other case, producers add substance to the solution to inhibit the corrosion effect of hydrogen peroxide (AmityHP75® by Amity International). Also in this case differences seems to be more commercial than technical, the efficacy of decontamination is due to the presence of hydrogen peroxide and the MCHP formation.

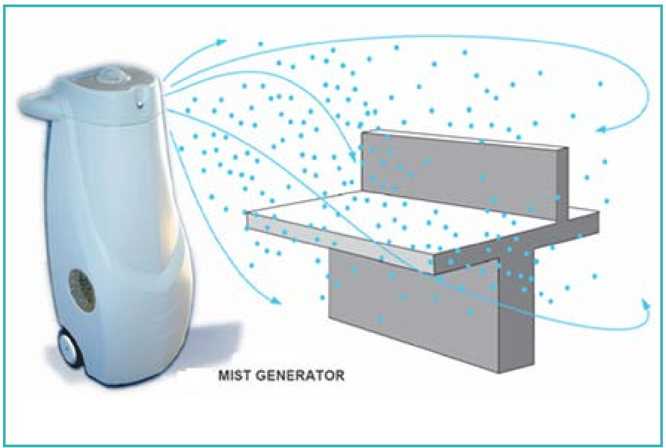


Fig.8: aHP generator: fine mist

VPHP and AHP are not responsible for the rapid sporicidal effect, they are merely the vehicle by which micro-condensed hydrogen peroxide is delivered to the surfaces. The bio-decontamination with hydrogen peroxide is a wet process, even if at micro-scale, because MCHP is not visible at naked eye. If there is no MCHP, there is no rapid sporicidal effect.

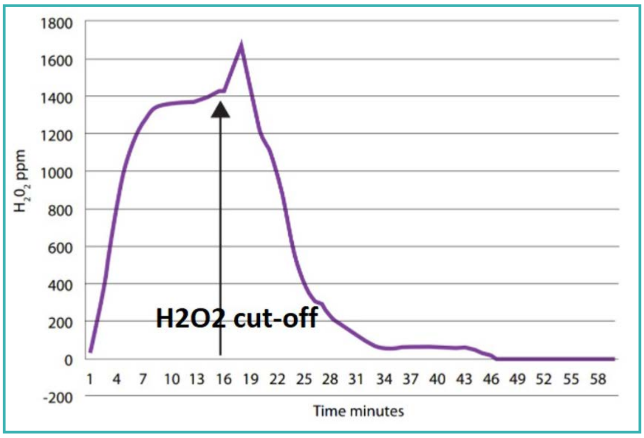


Fig.9: Hydrogen Peroxide Vapour concentration increases after H_2O_2 cut-off.

8. CONCLUSIONS

Despite both mechanisms (Vapour and Aerosol) achieve rapidly the bio-decontamination goal (6-log reduction), the two technologies have differences due mainly to the physical state of the decontaminant generated. Gases have a natural ability to occupy hard to reach areas. Even if a vapour is different from a natural gas, its behaviour is more like a gaseous substance than aerosol, and for this reason VPHP has a better homogeneous "three-dimensional" distribution into the environment than aHP. Micro-nebulization is visible at naked eye, the fine mist represents a frank wetting process. Therefore, aHP is less suitable than VPHP for electronics or sensitive to corrosion materials. Moreover, aHP cannot penetrate High Efficiency Particulate Air (HEPA) filters, while VPHP can pass through filters reticulates. On the other hand, aHP generator are more user-friendly and cheaper than VPHP generators. They have been conceived to be used by not expert personnel. The system needs to be placed inside the environment and turned on, without taking care about the environment conditions, as relative humidity and temperature. Moreover, aHP generator uses hydrogen peroxide solution with a concentration always less than 8%, which means less storage and transportation issues (in fact, under 8% hydrogen peroxide is classified only as an irritant agent). The question "which is better between VPHP or aHP?" requires the classic diplomatic answer "it depends!". The choice must be done considering several factor including: the environment geometry and physical constrain, the material to be decontaminate, the environmental conditions, the performance in term of log reduction and time, and of course the costs. So, if on the one hand VPHP offers a better distribution, repeatability, and performances, on the other hand aHP does not require strict environment conditions, it is easier to be used and installed, moreover it represents a lower money investment. To give the proper answer, it would be better to change the previous question in "Which method is more suitable for my application?". Vapour phase hydrogen peroxide can be considered more suitable for enclosure with limited volume, such as: pass-through chamber, material airlock, material transfer isolator, and passbox with dense, frequent and repeatable loads where strict documented validation is required. Aerosol Hydrogen Peroxide is more suitable for no-tight environments, not crowded with objects or equipment, such as lab rooms appropriately prepared.

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