

*Dekontamination von pharmazeutischen  
Isolatoren mit verdampftem  
Wasserstoffperoxid: Charakterisierung  
von Einflussparametern und  
Optimierung des Maschinendesigns*

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## Abbreviations

BAT	Biologischer Arbeitsstoff-Toleranzwert
BI	Biological Indicator
ca.	circa
CGMP	Current Good Manufacturing Practice
DFG	Deutsche Forschungsgemeinschaft
DPTE	Double Porte de Transfer Etanche
e.g.	for example
EN ISO	European Standard International Organisation for Standardisation
EPDM	Ethylene Propylene Diene
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
HEPA	High Efficiency Particulate Airfilter
HL	Humidity Level
h-x-diagram	Mollier-h-x-diagram for Moist Air
ISO	International Organisation for Standardization
ISPE	International Society for Pharmaceutical Engineering
MAC	Maximum allowable concentration
MAK	Maximale Arbeitsplatz-Konzentration
MOC	Materials of Construction
MPN	Most Probable Number
NIR	Near Infrared
PA	Polyamide
PC	Polycarbonate
PDA	Parenteral Drug Association
PE	Polyethylene
PIC/S	Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co- operation Scheme
PTFE	Polytetrafluoroethylene
PP	Polypropylene
PVC	Polyvinylchloride
PVDF	Polyvinylidene Fluoride
rH	relative Humidity
RTP	Rapid Transfer Port
SEM	Scanning Electron Microscope
SOP	Standard Operation Procedure
SPF	Specific Pathogen Free
SS	Stainless Steel
USP	United States Pharmacopeia
VDI	Verein Deutscher Ingenieure
VPHP	Vaporized Hydrogen Peroxide

# 1. General Introduction

## Aseptic Processing

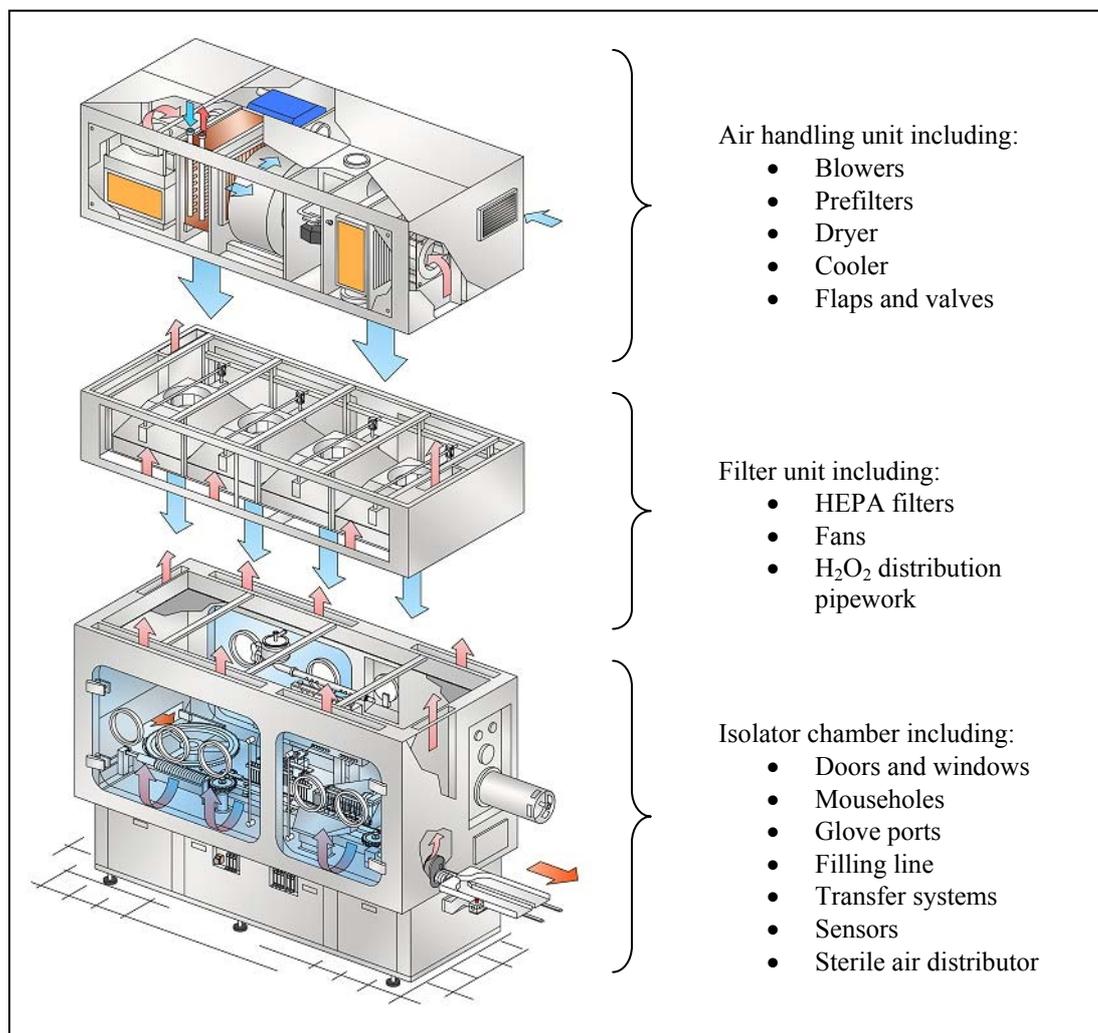
In the pharmaceutical industry several drugs and medicinal products have to be sterile when administered to the patient. These products include parenteral drugs like injectables which bypass the natural barriers of the body, but also other noninjectable preparations like ophthalmics and inhalants (Wallhäußer 1995). According to pharmacopeia these sterile forms have to be free of microbes to eliminate the potential infection risk when used on humans. In case the pharmaceutical ingredients cannot withstand terminal sterilization by moist heat or gamma irradiation, aseptic filling and packaging techniques have to be applied during manufacturing (Rauschnabel 2006). The percentage of products which cannot withstand terminal sterilization is constantly growing, mainly due to the increasing number of biopharmaceutical drugs. More than 80% of the sterile health care products are fabricated using aseptic processing (Wagner and Akers 1995).

Traditionally, aseptic production steps are conducted in cleanrooms with high air quality and unidirectional airflow. The environmental conditions in the production area have to be validated and critical parameters like the number of particles and microbial contamination are constantly monitored (PIC/S Annex 1 Manufacture of Sterile Medicinal Products 2009). Hygienic design and regular cleaning and sanitisation are required for machines and equipment located in the cleanroom. Before operators are allowed to enter the controlled area, they are subjected to strict disinfection and garment changing procedures (Wallhäußer 1995). Nevertheless, it is well-known that the operators are still the major source for microbial contamination in conventional cleanroom processing. Studies demonstrated that the risk for the release of contamination is correlated to the amount of operator interventions (Levchuk and Lord 1989, Luna 1986, Reinmueller 2000, Whyte 1994). To improve security and quality of sterile products, a new barrier technology called “isolator” was adapted and refined for the use in the parenteral pharmaceutical industry. The isolator technology incorporates all sterile filling operations into a small aseptic core zone and separates the operator from the process by the means of physical barriers (PIC/S Isolators used for Aseptic Processing and Sterility Testing 2007, FDA Guidance for Industry, Sterile Products produced by Aseptic Processing CGMP 2004).

## History of isolator technology

The first very basic barrier systems were used starting around 1800 for rearing germ-free animals. The barrier concept was further developed and improved until in the 1940s animals could be maintained continuously in the sterile environment (Wagner and Akers 1995, Reyniers and Trexler 1943). In the 1950s isolators were coming into use in nuclear technology for the handling of radioactive and hazardous materials. In this application, the main emphasis was on operator protection rather than product protection (Farquharson 1994a). Flexible isolators with gloves, half-suit and diving-suit constructions were utilized during this time. Towards the end of the decade, the first systems with chemical disinfection treatment were designed (Trexler and Reynolds 1957). In the beginning of the 1960s the double door transfer port DPTE (Double Porte de Transfer Etanche) was designed by La Calhene (Getinge la Calhene 2009) for application in nuclear industry. Now the transfer of material in and out of the controlled environment without compromising the integrity was easily possible. In

consequence of this development, also the pharmaceutical industry started using an adapted version of the previous isolator systems including air handling unit and sterilization equipment (Wagner and Akers 1995). The first applications in the pharmaceutical surrounding were flexible wall isolators for microbial monitoring, followed by product and operator protection applications in the 1970s. For bio-decontamination control, common methods at this time were the gassing with peracetic acid or formaldehyde. 1985 a flexible isolated aseptic filling line with DPPE port and glove technique was put into operation. End of the 1980s, the first rigid wall isolator for aseptic filling of ampoules with continuous transfer system and HEPA (high efficiency particulate airfilter) was started up. 1992 a syringe filling line with integrated hydrogen peroxide sanitisation was built (Farquharson 1994b). In the year 2000 the last gap in isolated aseptic processing steps was closed with a filling line for sterile powders including lyophilisation (Gail and Hortig 2004). Today, isolator technique represents the state-of-the-art technology in aseptic filling. More and more isolated fill lines come into operation.



**Figure 1. Schematic of pharmaceutical filling isolator (source: Robert Bosch GmbH)**

## Current definition of the term “isolator”

In general, barrier systems help to maintain sterility during an aseptic process or to protect the surrounding environment from harmful products. These barriers can be as simple as curtains in front of a machine or gloves worn by the operator. Depending on the degree of separation, the barriers provide a variable protection level for product and personnel, but they cannot totally eliminate the contact between the process and the operator or the exchange of air with the surrounding area (ISO / FDIS 14644-7 Draft 2004). The best separation is achievable with isolators, which also belong to the general group of barrier systems, but represent the most extreme form of segregation. In contrast to simple barriers, an isolator completely separates the process from the environment and offers the highest possible degree of protection against contamination for product and user. The schematic configuration of a pharmaceutical isolator is shown in Figure 1. Pharmaceutical production isolators as used today fulfill the following criteria as described in USP 32 <1208>, PDA Technical Report No. 34, Guidance for Industry, Sterile Products produced by Aseptic Processing CGMP 2004, Friedman 1998, Wagner and Akers 1995, Gail and Hortig 2004, and Meyer 1994:

- The operator is completely separated from the process by the means of gastight physical barriers, for example walls or windows. The barrier can be built from flexible plastic material or - prevailing in most aseptic applications - rigid wall with stainless steel corpus, firm plastic and glass boundaries.
- The user can only access the isolated process zone via gloves, half- or full-suits, but never directly.
- Air can only be exchanged with the environment via microbiological retentive filters (minimum grade HEPA: high efficiency particulate air filter) ensuring that personnel borne or air borne contamination cannot enter the isolated core zone or escape from the process.
- In aseptic processing, only active isolators are installed which use their own air handling unit for maintaining the defined maximum particle level, distribution and aeration of decontamination agent. Employed systems can use non-unidirectional turbulent or - more common in aseptic processing and especially in the critical area (e.g. filling and stoppering zones) - unidirectional air flow. The minimum particulate air classification inside the enclosure is ISO class 5 (EN ISO 14644-1 1999, Rauschnabel 2006, Proceedings of ISPE meeting San Antonio 2004, Guidance for Industry, Sterile Products produced by Aseptic Processing CGMP 2004). The surrounding area can be of lower air quality but is usually also controlled.
- For aseptic applications, all inner surfaces of the isolator can be treated with a chemical decontamination agent to reproducibly inactivate all viable bioburden prior to the start of the aseptic process. After decontamination, the germ-free environment on the inside of the isolator can be maintained for a long time allowing for extended production campaigns. Studies demonstrated that under worst case surrounding conditions, the aseptic environment can be maintained in the isolator for at least one month (Deguchi et al. 2003).
- Depending on the application, isolators can either be operated open or closed. In any case the isolator can be sealed for execution of routine leak tests and decontamination cycle.
- Material transfer in and out of the controlled enclosure is only performed via defined ports or interfaces.
- For open systems, continuous material transfer can be realized through so-called “mouseholes”. By maintaining a permanent overpressure inside the enclosure and a

constant overflow from the inside of the isolator to the surrounding area it is ensured that no air or contaminants can enter the system from outside. Through these validated small in- and outlets in the isolator wall, a controlled and uninterrupted supply of material, e.g. of vials or ampoules, is possible without compromising the isolator integrity (Guidance for Industry, Sterile Products produced by Aseptic Processing CGMP 2004).

- Discontinuous transfer of pre-sterilized material into and out of closed isolators proceeds aseptically using so called RTPs (Rapid Transfer Ports). Alternatively the transport can also take place via a second transfer isolator, an autoclave, hot air oven, e-beam tunnel or locks with chemical decontamination treatment.
- Isolators work under defined pressure difference relative to the environment. They can either be designed for containment only focussed on the protection of operators and the surrounding area from toxic substances. These containments are operated under negative pressure to prevent egress of material out of the system. Or the isolator is determined for aseptic processing with focus on the protection of sterility inside the isolated core zone and is therefore operated under positive pressure (PIC/S/S Isolators used for Aseptic Processing and Sterility Testing 2007, ISPE Baseline Sterile Manufacturing Facilities 1999).
- Containment isolators for aseptic processing have positive pressure in the core zone, with additional pressure zones to shield up- and downstream processing steps from contamination.
- Typically the parameters air velocity, differential pressure, temperature, and sometimes relative humidity are constantly monitored and alarmed when out of range.

### **Areas of application for isolator technology**

Today there are a lot of different areas of application for isolators in the pharmaceutical surrounding including the following:

- Sterile bulk filling of drugs and biopharmaceuticals (e.g. into bags, syringes, vials, ampoules)
- Quality control testing of pharmaceutical products in laboratory and production, in particular sterility testing of products
- Containment applications, for instance handling and production of cytotoxic materials, biologically active materials, category 3 and 4 pathogens, powder handling and transfer
- Specific pathogen free (SPF) and infected animal work in research laboratories
- Preparation of clinical supplies
- Pharmaceutical development and manufacturing of test batches for clinical trials for highly potent compounds
- Pharmaceutical manufacturing steps like weighing, dispensing, formulation, mixing, sub-division, sampling, final inspection with potentially high levels of airborne product particles
- Applications requiring an anaerobic or other specialized atmosphere (e.g. explosion-proof applications under inert gas)

In this work, the main focus is on pharmaceutical isolators for aseptic processing of medicinal products and their associated decontamination system.

## **Validation of decontamination methods**

For use as decontamination agent in the pharmaceutical industry, a sterilant has to meet several demands. The decontamination process has to be validated with a reproducible log-level inactivation of resistant organisms and it has to be biocidal against viable bacteria, spores, viruses, yeasts and fungi. The antimicrobial treatment of the inner surfaces of an isolator with hydrogen peroxide vapour prior to aseptic processing is rather a bio-decontamination procedure than a sterilization. According to the definition, decontamination is “a process that reduces contaminating substances to a defined acceptance level” (ISPE Baseline Sterile Manufacturing Facilities 1999) and that “eliminates viable bioburden via use of sporicidal chemical agents” (Guidance for Industry, Sterile Products produced by Aseptic Processing CGMP 2004). In contrast to that, parts which have direct product contact - like product containers or filling needles - have to be sterile prior to use. Sterilisation is described as “the act or process, physical or chemical, that destroys or eliminates all forms of life (e.g. microorganisms)” (ISPE Baseline Sterile Manufacturing Facilities 1999). To achieve sterility, these components can be autoclaved, steamed or treated by dry heat or irradiation.

To demonstrate the efficiency of a decontamination cycle and the pharmaceutical security of an isolator system, validation studies with representative microbial test challenges are carried out. Depending on the intended use and required sterility assurance, reduction of minimum 3 up to 6 log levels is requested (Sterile Products produced by Aseptic Processing CGMP 2004, VDI 2083 Draft 2009). Biological indicators (BIs) with high initial population, typically 10E6 spores of a resistant organism, are exposed to the decontamination process. Applied evaluation methods include half-cycle tests where BIs are inactivated in only half of the actual cycle time. Also overkill tests can be applied to show the complete kill of 10E6 or more spores. Bioburden methods assess the real level of initial contamination and resistance of occurring bioburden in the system and demonstrate kill accordingly. With fraction-negative studies, the cycle design is based on the necessary time to achieve a one log reduction of a resistant BI under the tested conditions.

## **Decontamination methods for pharmaceutical isolators**

In the early days of isolator utilization, several methods were employed and tested for microbial control inside of the isolated enclosure. In the beginning wiping the disinfectant was common. But due to the fact that wiping is hardly reproducible and is difficult to validate, this method was later replaced by spray and gassing procedures, which reach higher reduction rates and repeatable results. The decontamination agents are either atomized by the means of spray nozzles or evaporated before being introduced into the isolator (Belly and Wilkins 1998, Meyer 1994). Chemicals that were used most frequently for this purpose in the past include formaldehyde, ethylene oxide, peracetic acid, chlorine dioxide, ozone and hydrogen peroxide (Gail and Hortig 2004, Meyer 1994, Wagner and Akers 1995, USP 32 Sterility Testing – Validation of Isolator Systems). Most of these compounds were found to have unfavourable properties like high toxicity, corrosive impact on materials of construction (MOC), residue formation on surfaces, strong odour or negative environmental effects (Shintani 2009). Therefore today the prevailing method of choice for microbiological decontamination of pharmaceutical isolators is the gassing with vapour phase hydrogen peroxide VPHP (ISPE Baseline Sterile Manufacturing Facilities 1999, Gail and Hortig 2004, Rauschnabel 2006, Rios 2003).

## Hydrogen peroxide as decontamination agent

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is a transparent and water soluble liquid with characteristic acidic odour. In pure form it is stable, but when diluted it becomes unstable and degrades into the decomposition products water and oxygen. Several concentrations ranging from 3 up to 70%  $\text{H}_2\text{O}_2$  are commercially available, most of them containing stabilizers. Heat and contact with special materials rapidly accelerate the degradation process. Effective  $\text{H}_2\text{O}_2$  catalysers include most metallic substrates (e.g. iron, copper, and manganese), alkalines, organic molecules or enzymes like catalase or peroxidase. Hydrogen peroxide is a weak acid, and in concentrated solution it acts as a strong oxidising agent (Wallhäüßer 1995, McDonnell 2007, McDonnell and Russel 1999, Block 2001).

Hydroxyl radicals were shown to be the reactive species which causes the antimicrobial effect (Clapp et al. 1994, Keyer et al. 1995). This is analogous to the effect of ionizing radiation, which is also believed to be an effect of oxidation by hydroxyl radicals (Peloux et al. 1962, Powers et al. 1972). The mechanism of action involves the irreversible oxidation of essential cell components. Gould and Hutchins 1963 suggested that especially exposed sulfhydryl groups and double bonds in proteins are attacked by the radicals. This causes denaturation of enzymes, RNA, DNA and damages in membrane lipids. It was discovered that the addition of non-toxic amounts of metal ions can intensify antimicrobial action of peroxide compounds by accelerating decomposition and formation of hydroxyl radicals (Dittmar et al. 1930, Yoshpe-Purer and Eylan 1968, Bayliss and Waites 1976, Imlay et al. 1988, Sagripanti 1992, Akers et al. 1995, Akers and Kokubo 2002). For use as sterilant, hydrogen peroxide is considered to have low toxicity. In high concentrations the liquid form is irritating to the skin and causes inflammation of the respiratory system when inhaled in the vaporous form (MAC: maximum allowable concentration at the working place is 0.5 ppm; DFG MAK- und BAT-Werte-Liste 2009). When concentrations of more than 100 ppm  $\text{H}_2\text{O}_2$  are inhaled, severe damage can be caused in the respiratory system. But in industrial practice, occupational safety measures are well manageable. Hydrogen peroxide is noncarcinogenic and nonmutagenic and due to its harmless decomposition products it leaves no residues on surfaces and is environmentally friendly (Block 2001).

The compound hydrogen peroxide was first discovered and examined by the French chemist Thenard in 1818 and its antimicrobial properties have been known since it was used as a disinfectant by B.W. Richardson in 1858 (Block 2001). In connection with its use as preservative agent for food and beverage products, the inactivation characteristics of liquid hydrogen peroxide towards food associated microorganisms were started to be investigated (Schrodt 1883, Heinemann 1913, Wilson et al. 1927, Yoshpe-Purer and Eylan 1968, Naguib and Hussein 1972). Later, the inactivation characteristics of liquid hydrogen peroxide towards various other microbial forms and species was studied in detail by several researchers. It was observed that liquid  $\text{H}_2\text{O}_2$  has a broad spectrum efficacy against viruses, bacteria, fungi, yeasts and bacterial spores (Mentel and Schmidt 1973, Toledo et al. 1973, Wardle and Renninger 1975, Buchen and Marth 1977, Sagripanti et al. 1997, Wallhäüßer 1995, Pottage et al. 2009). Anaerobes which do not produce catalase show lower resistance towards the sterilant than aerobic bacteria (Block 2001). Compared to vegetative cells bacterial spores were found to be most resistant towards the inactivation with hydrogen peroxide (Toledo et al. 1973, Ito et al. 1973, Cerny 1976, Rutala et al. 1993). In general, improved microbicidal action was found for increasing sterilant concentration and rising temperature of the test solution (Curran et al. 1940, Toledo et al. 1973, Cerny 1976, Smith and Brown 1980, Wilke 1992). Wilke (1992) noticed a flattening of the inactivation curve when liquid  $\text{H}_2\text{O}_2$  concentrations  $\geq 20\%$  were used. Swartling and Lindgren (1962, 1968) observed a lag phase

followed by first order inactivation kinetics during their studies on aseptic filling and sterilization of food packaging material with liquid hydrogen peroxide. Similar shaped survival curves were obtained by Toledo et al. (1973), who also noticed an enhanced kill for dry spores compared to wet spores. Beyond that, the results of Cerf and Hermier (1972) showed an additional tailing of the inactivation behaviour towards the end of the exposure to the sporicidal agent. The tailing was later interpreted by Cerf and Metro (1977) to be caused by lump formation of spores resulting in protection of single microorganisms by the catalase contained in adjacent spores. The resistance of wet and dry spores respectively was found to be depending on the applied temperature by Smith and Brown (1980).

## **Decontamination with vaporized hydrogen peroxide (VPHP)**

An important step in the history of hydrogen peroxide as a sterilant was done when it was discovered that at low temperatures the antimicrobial effectiveness of hydrogen peroxide in the gaseous form is even higher than in liquid form. In 1976 Huber inferred from experiments with decontamination of packaging materials with liquid hydrogen peroxide, that during drying phase non-wetted parts of the packaging foil were decontaminated by vaporized hydrogen peroxide (VPHP). Moore and Perkinson (1979) described in detail the process of “cold” gas sterilization at temperatures  $< 80^{\circ}\text{C}$  with vaporized hydrogen peroxide. In several test systems they showed the sporicidal action of vaporized hydrogen peroxide against spores of *Bacillus subtilis* and *Bacillus stearothermophilus*. The concentrations in the gas phase required are noticeable less than in the liquid form to achieve equivalent kill (Wilke 1992) which is assumed to be due to the phase change of the peroxide. The higher effectiveness in the gas phase also results in shorter treatment periods. With rising concentration in the gas phase, Wilke (1992) detected improved inactivation characteristics of the VPHP. Operating conditions ranging from 4 up to  $80^{\circ}\text{C}$  with sterilant concentrations from 0.5 to 10 mg/L were shown to be sporicidal; in practice VPHP is typically applied at temperatures between  $35 - 49^{\circ}\text{C}$  and in concentrations ranging from 0.2 to 2 mg/L (140 to 1400 ppm) (Shintani 2009, Bioquell 2009). Different temperatures were tested especially for the use in VPHP decontamination of pharmaceutical isolators and filling equipment. Ambient temperature was concluded to be most advantageous for this application (Wilke 1993). The activity of vaporized hydrogen peroxide against a wide range of different microorganisms including gram-negative and gram-positive bacteria, yeast, fungi, viruses and bacterial spores was demonstrated by several researchers (Wang and Toledo 1986, Rickloff and Orelski 1989, Heckert et al. 1997, Block 2001, Johnston et al. 2005).

In conformity with Spaulding’s classification of resistance (Spaulding 1972) spores were found to be the most resistant microbial forms. Rickloff and Orelski (1989) showed that spores of *Geobacillus stearothermophilus* were most resistant towards VPHP and are therefore typically used as test organisms for microbial challenge of decontamination procedures with VPHP in the pharmaceutical industry. Today, industrial fields of application for VPHP include surface treatment in aseptic food and pharmaceutical processing (e.g. decontamination of sterility testing or manufacturing isolators or containment devices, packaging material in food filling lines). Also the gassing of whole rooms with VPHP (e.g. in research facilities or laboratories) is common today (USP 32 <1035> Biological indicators for Sterilization, Block 2001, French et al. 2004, Engelhardt 2005, Hall et al. 2007, Boyce et al. 2008). The potential use of vaporized hydrogen peroxide for decontamination of non heat-resistant medical devices (e.g. endoscopes, dental materials) and laboratory equipment (e.g. freeze-dryers, centrifuges) is currently under evaluation (Shintani 2009, McDonnell 2007, Klapes and Vesley 1990, Heckert et al. 1997).

## Decontamination cycle with vaporized hydrogen peroxide

For surface decontamination with vaporized hydrogen peroxide the aseptic processing isolator or other enclosure is connected to a gas generator. Here, the liquid hydrogen peroxide solution (e.g. 35%) is transformed from liquid to gas phase by flash evaporation. The gaseous H<sub>2</sub>O<sub>2</sub> is mixed with a carrier stream of dry air and transported under controlled conditions into the interior of the isolator passing the retentive HEPA filters. Depending on the application, the decontamination cycle can be performed at ambient pressure or in vacuum mode to enhance penetration into covered or occluded areas. A standard decontamination cycle with VPHP consists of the following four sequential phases:

- I. Preparation phase: heating of ductwork and reduction of relative humidity inside the isolator to typically ca. 20% rH.
- II. Conditioning phase: introduction of vaporized hydrogen peroxide at high rate until defined level of H<sub>2</sub>O<sub>2</sub> concentration is reached inside the chamber.
- III. Decontamination phase: introduction of H<sub>2</sub>O<sub>2</sub> gas at reduced rate to maintain hydrogen peroxide at defined concentration level until inactivation period is finished
- IV. Aeration phase: removal of hydrogen peroxide from the isolated system (e.g. from air, surfaces, filters, ductwork etc.) by rapid dilution with high amount of fresh HEPA filtered air to predefined concentration level (e.g.  $\leq 1$  ppm H<sub>2</sub>O<sub>2</sub>, according to product and application).

Depending on isolator size, load, geometry and air change rate a decontamination cycle takes typically between 3 and 8 hours, whereas the aeration is the most time consuming part. Up to date processing systems keep the bio-decontamination cycle time below 4 hours.

## VPHP decontamination – current state of knowledge

Although VPHP is utilized for diverse industrial purposes today, the mechanism of action and the parameters influencing the decontamination results are not fully understood and clarified until now. It is of essential importance to evaluate and define conditions, which ensure robust and reliable decontamination processes in practice. Cycle parameters which are known to have an effect on the microbial inactivation include hydrogen peroxide concentration, humidity, temperature, cycle length, flow conditions and distribution of the sterilant. Furthermore, as the decontamination with VPHP is considered a surface treatment, also the material characteristics and geometry of the object to be sterilized have an impact on the result.

### *Hydrogen peroxide concentration*

It is agreed among most scientists that an adequate hydrogen peroxide concentration in the gas phase is required and represents a crucial factor for the decontamination success with VPHP. Several studies found enhanced inactivation characteristics for test organisms with growing peroxide concentration (Graham et al. 1992, Wilke 1992, Block 2001, Steris Cycle development Guide 2001, Engelhardt 2005, Hultman et al. 2007). Wilke (1992) demonstrated that for low levels of hydrogen peroxide in the gas phase no microbicidal action could be detected on packaging material inoculated with  $\geq 10E5$  spores of *B. subtilis*. For concentrations equal to or greater than 0,28 Vol% he found a rising inactivation rate with growing concentration. When reaching a concentration of  $\geq 0,6$  Vol% he detected a flattening

of the inactivation curve, the kill could not be further increased with higher peroxide concentration. However, Imai et al. (2006) doubt the direct correlation between concentration of hydrogen peroxide in the gas phase and the achieved kill characteristics. Also the absolute H<sub>2</sub>O<sub>2</sub> concentration level necessary to obtain adequate kill varies in the results of different studies. For example, Sigwarth and Stärk (2003) presented 10E6 inactivation of *Geobacillus stearothermophilus* spores achieved with only ca. 350 ppm H<sub>2</sub>O<sub>2</sub> during decontamination phase. Due to its influence on the sterilization process, the hydrogen peroxide concentration is usually monitored during the decontamination run. Commonly applied methods are monitoring by the means of a near infrared (NIR) spectrophotometry or measuring with chemical sensors (Brown et al. 1998, Corveleyn et al. 1997, Näther et al. 2006).

### *Hydrogen peroxide distribution*

Because decontamination with vaporized hydrogen peroxide is a surface treatment, a uniform distribution of the sterilant and exposure of all critical surfaces is of particular importance. The flow pattern inside the enclosure to be sterilized is by default tested during cycle development. In case dead spots are detected, the distribution can be improved by the means of fans to ensure satisfactory circulation and constant kill rates in all regions (ISPE Baseline 1999, Guidance for Industry, Sterile Products produced by Aseptic Processing CGMP 2004).

### *Hydrogen peroxide cycle duration*

In general, the microbicidal action of the VPHP increases with growing exposure time. The duration of the decontamination step has to be defined as compromise between effectiveness of the sterilant, compatibility with the construction materials and the overall cycle time (Wilke 1992, VDI 2083 Draft 2009, PDA Technical Report No. 34 2001).

### *Temperature during hydrogen peroxide exposure*

Regarding the effect of temperature on the VPHP inactivation, most published literature describes an increased kill effect for rising temperatures (ISPE Baseline 1999, Block 2001, Engelhardt 2005). It is hypothesized that the improved kill of the test spores can be attributed to a synergistic effect of heat and hydrogen peroxide. The hydrogen peroxide could sensitize the spores so that they are more susceptible to the heat treatment. For application in sterilization of packaging material in aseptic food processing, the VPHP is usually applied at elevated temperatures of ca. 80°C with only some seconds contact time (Block 2001). Wilke (1992) conducted extensive studies regarding VPHP inactivation of *B. subtilis* spores on packaging material at different temperatures. He described three different temperature ranges with different behaviour of the sterilant. At temperatures of ca. 60°C he described effective microbial kill which he attributes to the action of condensation and re-evaporation during drying step. Also the temperatures from 110°C - 120°C are reported to be highly active due to the action of gaseous H<sub>2</sub>O<sub>2</sub>. On the contrary, the temperature range between 70°C and 90°C is characterized as transition phase between condensation driven and mainly gaseous activity of the sterilant which is less microbicidal. The author emphasizes the thorough optimisation which is required for the VPHP process. However, for VPHP decontamination of pharmaceutical isolators the contact times are considerably higher and the prevailing temperature range is at or slightly above room temperature. In contrast to the results described before, Klapes and Vesley (1990) found better kill for lower temperatures when gassing a

centrifuge with VPHP. In the chamber of the equipment (at temperature 4°C) 10E6 *Bacillus subtilis* spores were inactivated within 8 min, while for the spore carriers placed in the outer gap (at temperature 27°C) a 32 min decontamination cycle was needed to reach full kill of the same initial spore count. The scientists explained their findings with faster hydrogen peroxide decomposition due to higher surface area and temperature. But as positives were found mainly in the positions most distant from H<sub>2</sub>O<sub>2</sub> injection port, it is also discussed that the effect might be caused by poor distribution of the sterilant.

#### *Humidity and condensation during hydrogen peroxide exposure*

When it comes to VPHP decontamination, one of the subjects discussed most controversial is the influence of humidity on the process and the debate whether the microbial inactivation is a dry process or driven by condensation. A group of scientists claim the sporicidal effect to be based on the interaction of gaseous hydrogen peroxide with the microorganisms. In this approach the formation of condensate is preferably avoided to prevent unfavourable side effects like material corrosion, non-uniform inactivation or prolonged aeration times (Graham et al. 1992, Akers et al. 1995, Hultman et al. 2007). Engelhardt (2005) reported even a retarded kill kinetic for inactivation of spores on packaging material when increasing the absolute water content in hot peroxide loaded air. Opposing to that other researchers believe the deposition of a sterilant condensation film of  $\leq 1 \mu\text{m}$  on the surfaces, the so-called highly concentrated “microcondensation” ( $> 70\% \text{ H}_2\text{O}_2$ ), to be responsible for the microbicidal action of the VPHP. They bring forward the argument that under standard decontamination conditions (regarding applied hydrogen peroxide concentration, relative humidity and temperature) the formation of condensation can thermodynamically not be avoided (Marcos-Martin et al. 1996, Watling et al. 2002, Watling 2002). Depending on the temperature the air can hold a defined amount of humidity. When the H<sub>2</sub>O<sub>2</sub> and water concentrations are rising, at a certain stage the dew point will be reached and condensation starts to form. In particular the conditions at near-saturation level are believed to be especially sporicidal. In a recent publication Radl et al. (2009) provided an h-x diagram and computational programs for simplified prediction of condensation phenomena in VPHP decontamination chambers.

#### *Materials of construction for enclosures to be decontaminated with VPHP*

When designing an isolator system for decontamination with VPHP one of the most important decisions is the selection of the materials of construction (MOC). Besides the development of cycle parameters for the decontamination process also the materials of the enclosure can influence cycle time and performance of the decontamination system. Today, frequently applied materials for the main components include 1.4435, 1.4571 and 1.4404 stainless steel and glass. Alternatively, windows can also be made of polycarbonate or acrylic plastic. Well-established materials for gloves are chlorosulfonopolyethylene (Hypalon<sup>TM</sup>), neoprene, butyl rubber or equivalently coated materials. In general gaskets can be made of viton, silicone rubber or EPDM seals. For component parts inside the isolator, metallic materials like titanium, hastelloy or anorganic non-metallic materials like ceramic, quartz, enamel substrates can be used. Sometimes anodized aluminum is applied, typically for the fabrication of cooling coils. Plastic materials that are commonly used e.g. for equipment parts of filling lines and transport systems contain PTFE, PVDF, PETP, POM, PP, PC, PA6 and to some extent also PVC or PE. Filters typically consist of glass fibres mounted in stainless steel or aluminum frames (Wagner and Akers 1995, PDA Technical Report No. 34 2001, Gail and Hortig 2004, Reiber and Schenk 2006). For the texture of stainless steel surfaces inside the isolator a

smooth finish, polished or even electro-polished for parts with extraordinary high quality requirements, is generally recommended. Seams should be fully welded and hygienic design applied, e.g. with round corners for easy cleaning and no cracks and crevices (Wagner and Akers 1995, ISPE Baseline Volume 3 1999, Williams 2004).

The selection of MOC used for VPHP decontaminated pharmaceutical barrier systems is based on the following basic requirements:

- Physical stability and strength of the materials is required during standard handling and process operations.
- All construction materials have to be chemically resistant and compatible with the intended products, cleaning and sporicidal agents used for the decontamination process. It is reported that compared to the liquid phase, hydrogen peroxide is less aggressive towards materials when applied in the gas phase with typical concentrations ranging from 500 ppm to maximum 2000 ppm H<sub>2</sub>O<sub>2</sub>. Materials that are regarded critical in terms of chemical stability against VPHP include especially gloves, natural rubber and nylon products (Wagner and Akers 1995).
- The construction materials must not catalyse the degradation of the sterilant H<sub>2</sub>O<sub>2</sub> (ISPE Baseline Volume 3 1999, Gail and Hortig 2004).
- The absorption of the VPHP into the MOC is an important factor and should be minimized as far as possible. It is known that plastic materials, e.g. PVC or silicone, absorb H<sub>2</sub>O<sub>2</sub> and degas during aeration, thus prolonging the overall cycle time significantly. Where possible, these materials should be replaced by stainless steel and glass or other materials with proven low sorption rates (Wagner and Akers 1995, Gail and Hortig 2004).
- Materials have to show proper cleanability.

Lately the ability of different construction materials to be decontaminated by VPHP is gaining increasing attention. Several studies emphasize the influence of the MOC on the decontamination success with VPHP. Sigwarth and Stärk (2003) reported varying resistance of spores towards VPHP decontamination when inoculated onto carriers made of different MOC. They found significantly increased D-values<sup>1</sup> for some tested materials which could be reproduced in several test isolators. The researchers concluded that the varying resistance of the spores was caused by differences in the surface structure and physico-chemical characteristics of each MOC. It is well-documented in literature, that materials with extraordinary rough, porous surfaces or cellulosic materials with meshed texture should generally be avoided in isolators due to their structure which can protect microorganisms from the exposure to the gaseous sterilant (Wagner and Akers 1995, Rogers et al. 2005). Akers and Kokubo (2002) also found some differences in the decontamination behaviour of materials, but they presume that inoculation effects might be the prevailing reason for the variations. They carried out studies with 10E3, 10E4 and 10E5 inoculated plastic carriers for *B. subtilis*, *B. stearothermophilus* and *Chaetomium globosum* and described retarded kill for increasing spore concentrations on the test carriers. Their explanation for the observed effect was the growing protection of the test organisms from the vapour at higher spore concentrations. Engelhardt (2005) found faster kill and no tailing for carriers inoculated with ethanol based spore suspension compared to carriers treated with water based suspension when subjected to the same decontamination process. He traced the effect back to the fact that ethanol has a lower surface tension than water. This might result in a better spore distribution on the carrier during inoculation procedure and therefore in reduced formation of lumps which can protect

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<sup>1</sup> D-value is the time (in minutes) of exposure at given killing conditions, which causes a one log reduction of the population of a specific microorganism (Guidance for Industry 2004)

spores on their inside. Although some information is available in literature, no systematic and statistically sound investigation on the influencing factors of material properties, effects of inoculation or surface finish of materials on decontamination success with VPHP has been performed so far. This is also reflected in documents from authorities which recommend the testing of isolator materials and their texture with regard to decontamination issues (Guidance for Industry, Sterile Products produced by Aseptic Processing CGMP 2004, VDI 2083 Draft 2009).

### *Influence of complex geometrical structures on the decontamination with VPHP*

Since the decontamination with VPHP is a surface treatment with limited penetrating characteristics, one of the most challenging applications is the decontamination of complex structures or surfaces that are occluded or difficult to access by the sterilant. In isolator design it is essential to avoid such dead spaces and gaps as far as possible - especially during decontamination. But in some applications, complex arrangements cannot be totally eliminated. One example is the growing implementation of automation technology into barrier systems, e.g. robotic arms with the mechanical parts covered by bellows. Also the development of VPHP applications for the decontamination of process equipment or rooms involves decontamination of complex structures and geometries (Klapes and Vesley 1990, Graham et al. 1992, Johnson et al. 1992, French et al. 2004, Hall et al. 2007). The process of H<sub>2</sub>O<sub>2</sub> penetration into trenches and dead ends is a mixture of passive diffusion and active convection (Opfell et al. 1964). At atmospheric pressure, the distribution of the sterilant can be intensified by the means of fans, which are placed in critical locations or by forced convection through pipes to be decontaminated (Mau et al. 2004). Graham et al. (1992) found, that the generation of vacuum can support the diffusion of the VPHP into narrow lumens, but this method is not applicable for all applications in praxis. Altogether, there is only limited information available regarding the gap penetration capability of VPHP and the impact of the geometrical design of chamber and equipment to be sterilized.

## **Experimental Setup**

For this work a pharmaceutical rigid wall test isolator, originally intended for aseptic filling operations, was placed in a class D laboratory environment and used to carry out different trials regarding the inactivation of microorganisms with VPHP. Prior to injection of the vaporous sterilant, the test atmosphere was reproducibly dehumidified to ca. 20 % starting relative humidity. A hydrogen peroxide generator was connected to the enclosure system for flash evaporation of hydrogen peroxide. For the analysis of effects caused by different humidity levels in the isolator, the additional evaporation of water simultaneous to gassing with hydrogen peroxide was possible. VPHP decontamination test cycles with different sets of process parameters to vary hydrogen peroxide concentration, humidity, condensing conditions and cycle length were developed. Cycle parameters temperature, relative humidity, hydrogen peroxide and water concentration, condensation, differential pressure, and air velocity were constantly monitored with sensors placed inside the barrier system. The challenge for the decontamination system has to be verified by the means of biological indicators (BIs), that respond to the adjusted inactivation conditions. For each set of cycle parameters the respective sporicidal activity was characterized using BIs inoculated with defined initial populations of highly resistant *Geobacillus stearothermophilus* spores. This is the common test organism for VPHP installations in the pharmaceutical environment. Commercially available standard stainless steel carriers as well as material carriers made of a

variety of isolator MOC and fabricated especially for this purpose, were tested for their decontamination characteristics. For the investigation of the gap penetration capability of VPHP a stainless steel test model was employed, where commercial biological indicators could be placed at the rear end of gaps with variable height and depth. The gap model was prepared with BIs and exposed to VPHP decontamination cycles inside the isolator. Most probable number (MPN) (Cochran 1950, Alexander 1982, Sun et al. 2006) and fraction-negative microbial methods (DIN EN ISO 11138-1 2006) were used to assess the inactivation kinetics for each experimental setup.

## Objectives

Although VPHP is used for multiple applications in the pharmaceutical and adjacent industries, among experts there is no uniform understanding regarding the mechanism of action and the optimum process parameters of the decontamination technology. Because of the sensitive field of application, growing number of aseptic VPHP process installations as well as the increasing range of applications for VPHP (e.g. decontamination of rooms and equipment) further research is needed to clarify the fundamental processes. In this thesis the focus is on the VPHP decontamination of pharmaceutical isolators for aseptic filling procedures. Target criteria were the deepening of basic knowledge about the inactivation of microorganisms with VPHP and an optimisation of hydrogen peroxide decontamination cycles based on the gained data. The aim of this work was to systematically investigate the effect of different process parameters and isolator design related factors like material selection and penetration of sterilant into defined geometrical structures. In detail the objectives were:

- Assess and quantify the influence of process parameters hydrogen peroxide and water concentration, relative humidity, and dwell time onto the microbial inactivation kinetics
- Investigate in detail the sporicidal effect of occurring condensation during VPHP gassing compared to dry decontamination cycles
- Test different isolator construction materials regarding resistance behaviour of inoculated test organisms.
- Determine influence of surface finish and physio-chemical material properties that have an impact on the decontamination behaviour of microbial challenge inoculated on surfaces
- Study inoculation effects on VPHP decontamination characteristics
- Examine the microbial reduction with VPHP in gaps depending on defined height and depth of the tested interstices and define design limits for geometries which can be penetrated and decontaminated with gaseous hydrogen peroxide
- Analyze the influence of the factors hydrogen peroxide and water content in the sterilant atmosphere onto the penetration characteristics of VPHP into small lumens
- Derive practical guidance recommendations for optimisation of design and process parameters for systems decontaminated with VPHP for the pharmaceutical and related industries

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## General Introduction

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## 2. Suitability of Different Construction Materials for Use in Aseptic Processing Environments Decontaminated with Gaseous Hydrogen Peroxide

### Abstract

The presented study examined the suitability of 49 different materials for the decontamination with vaporized hydrogen peroxide (VPHP). The test pieces were derived from materials commonly used in the construction of aseptic pharmaceutical machines and installations. Biological indicators manufactured from original material samples and inoculated with  $10^6$  spores of *Geobacillus stearothermophilus* spores were exposed to defined VPHP decontamination cycles. By the means of D-value calculation for the different materials the inactivation behaviour of the spores depending on the material properties was investigated. The results show that the material characteristics have an impact on the time required to inactivate the initial microbial population on the different materials. In particular material properties like roughness and wettability seem to affect the VPHP decontamination success and were studied in detail. Best and worst case materials were identified and recommendations developed for the selection, manufacture and surface treatment of suitable materials for the application in aseptic environments decontaminated with gaseous hydrogen peroxide.

Paper published:

Unger, B., Rauschnabel, U., DÜthorn, B., Kottke, V., Hertel C., and Rauschnabel, J. 2007. Suitability of Different Construction Materials for Use in Aseptic Processing Environments Decontaminated with Gaseous Hydrogen Peroxide. PDA Journal of Pharmaceutical Science and Technology, 2007, 61(4): 255–75.

### **3. The Influence of Humidity, Hydrogen Peroxide Concentration, and Condensation on the Inactivation of *Geobacillus stearothermophilus* Spores with Hydrogen Peroxide Vapor**

#### **Abstract**

The purpose of this study was to examine factors influencing the effectiveness of decontamination cycles with vaporized hydrogen peroxide (VPHP). Biological indicators inoculated with  $10^6$  spores of *Geobacillus stearothermophilus* were exposed to decontamination atmospheres with different levels of hydrogen peroxide concentration, relative humidity and condensation. The sporicidal activity of the respective VPHP decontamination environment was evaluated by the means of calculated D-values. It was demonstrated that the determining factor for the microbial inactivation is the molecular deposition of water and hydrogen peroxide on the exposed surfaces. Subvisible condensation was shown to be necessary for the inactivation of the spores. For low hydrogen peroxide concentrations the inactivation was found to be enhanced by an increasing water concentration in the decontamination chamber. The results prove that with increasing hydrogen peroxide concentration the inactivation becomes independent from the humidity in the test atmosphere.

Paper published:

Unger-Bimczok, B., Kottke, V., Hertel C., and Rauschnabel, J. 2008. The Influence of Humidity, Hydrogen Peroxide Concentration, and Condensation on the Inactivation of *Geobacillus stearothermophilus* Spores with Hydrogen Peroxide. *Journal of Pharmaceutical Innovation*, 2008, 3: 123-33.

## 4. Hydrogen Peroxide Vapor Penetration into Small Cavities during Low-Temperature Decontamination Cycles

### Abstract

The objective of the paper was to study the capability of vaporized hydrogen peroxide (VPHP) to penetrate and decontaminate narrow cavities and gaps. A test piece with adjustable variable height, depth and material of the cavities was used to expose biological indicators inoculated with spores of *Geobacillus stearothermophilus* at the rear end of the gaps. The test apparatus, prepared with the microbial test organisms, was exposed to VPHP decontamination cycles with varying cycle length, hydrogen peroxide and water concentration. It was demonstrated that the decisive factor for the inactivation of the spores by the penetrating VPHP was the ratio of height and depth of the cavities. Varying gap heights and depths were examined and geometrical limitations for a successful microbial inactivation were specified. It is shown that the decontamination in narrow cavities and the penetration of complex structures can be enhanced by a high peroxide concentration and extended cycle time.

Paper accepted for publication:

Unger-Bimczok, B., Kosian, T., Kottke, V., Hertel C., and Rauschnabel, J. 2011. Hydrogen Peroxide Vapor Penetration into Small Cavities during Low-Temperature Decontamination Cycles . Journal of Pharmaceutical Innovation, 2011.

## 5. Summary

In the pharmaceutical industry sterile drugs, which can not be terminally sterilized, have to be prepared and handled under aseptic conditions. Traditionally, the aseptic processing of the drugs was performed in clean room areas with high room classification. To improve product security, the application of isolator technology with physical separation of the process from the environment and the operator is a popular alternative. Prior to the aseptic processing, the inner isolator surfaces have to be treated with a chemical sterilant to reduce the microbial contamination to a defined acceptable level. Today vaporized hydrogen peroxide (VPHP) is most commonly used for this purpose. A typical decontamination cycle with VPHP is composed of the phases preparation, conditioning, bio-decontamination and aeration. Different parameters like hydrogen peroxide concentration, humidity, and condensation have an influence onto the microbicidal activity of the decontamination cycle. Also isolator design factors like material of construction and geometrical structure of the chamber can impact the inactivation results. Although VPHP is widely used in the pharmaceutical and associated industries, no uniform understanding of the mechanism of action as well as overall recommendations for optimized cycle parameters are available in literature up to now. The objective of the presented thesis was to investigate in detail the mode of action of the VPHP and the relationship between different influencing cycle parameters in order to develop a recommendation for optimum decontamination conditions. An additional goal was to analyze the impact of different construction materials, surface finish and geometrical structures onto the inactivation efficiency of the sterilant to improve the design of aseptic processing machines regarding VPHP decontamination. A detailed literature review with reference to the current state of knowledge regarding VPHP decontamination is presented in the introduction part of the thesis.

For the studies an 8 m<sup>3</sup> pharmaceutical test isolator connected to a VPHP generator was used. Standard decontamination cycles with varying combinations of hydrogen peroxide and water concentration, cycle time and condensation levels were developed. Biological indicators (BIs) with defined initial spore population of *Geobacillus stearothermophilus* were exposed to the different VPHP cycles. These organisms are known for their notably high resistance towards gaseous hydrogen peroxide and are the standard test germ for validation of VPHP cycles in the pharmaceutical industry. By determination of inactivation kinetics for the microbial test challenge, the sporicidal activity for each set of cycle conditions was evaluated. The applied microbial methods were enumeration of surviving spores after defined exposure by Most Probable Number (MPN) technique as well as the determination of decimal reduction times (D-values). BIs were not only tested when openly exposed to the sterilizing atmosphere, but also inside of defined gaps to challenge the penetration capability of the VPHP into small lumens under diffusive conditions. Different construction materials were inoculated with defined spore populations to investigate the resistance behaviour of the spores on varying surfaces. Supplementary the physico-chemical characteristics of the respective materials were analyzed in detail to draw conclusions regarding correlation of surface quality and inactivation properties.

The results demonstrate that different cycle parameters influence the sporicidal activity of the VPHP. Furthermore the results show that the decisive factor for a successful decontamination is the overall microscopic interaction with the bioburden on the surface. But due to the fact that this interaction is hard to quantify the most important macroscopic parameter has to be taken into focus: the H<sub>2</sub>O<sub>2</sub> gas concentration. The determined results and the suggested model incorporate and explain all previously published literature which seemed to be partially

controversial. It is demonstrated, that the microcondensation in the sub-visible range is effective for good inactivation performance and that further condensation in the visible range does not enhance the microbicidal activity. The data illustrate that in case all other parameters are kept constant, the microbial inactivation is accelerated by increasing hydrogen peroxide concentration. An H<sub>2</sub>O<sub>2</sub> level of 800 ppm ensures a sufficient deposition of sterilant onto the surface and results in excellent and reproducible kill. For sterilant levels increasing 800 ppm no further improvement in inactivation is detectable. It is shown that for openly exposed BIs a lower hydrogen peroxide concentration (400 ppm) can be compensated by higher humidity. The elevated water content in the decontamination atmosphere promotes the sterilant deposition. The higher the hydrogen peroxide level is, the more independent from humidity becomes the inactivation effect. For H<sub>2</sub>O<sub>2</sub> levels in the range of 800 ppm, the microbicidal activity of the VPHP is found to be independent from the water concentration.

In contrast to the openly exposed BIs, for the inactivation of spores exposed under diffusive conditions inside of gaps, a lower hydrogen peroxide level can not be compensated by higher humidity. Solely the hydrogen peroxide concentration and the overall cycle duration are able to influence the decontamination success inside of the trenches. It is demonstrated that in principle complex structures can be decontaminated by the means of VPHP but the penetration capability is limited. The inactivation is impeded with decreasing gap cross section and with increasing gap depth. If possible, occluded surfaces and small lumens should be avoided when manufacturing aseptic processing systems. When this is not possible, certain design criteria have to be considered (gap height > 4 mm, gap depth < 30 mm). Transferability of the data generated by the means of the gap model onto real construction elements is successfully demonstrated. It is shown that different construction materials and surface textures have an impact onto the resistance behaviour of spores towards VPHP. Worst and best case materials are identified and potential reasons for the unlike performance are determined. Rough, in particular highly porous surfaces, are shown to partially protect exposed microorganisms from the sterilant, so that reproducible decontamination can not be ensured within reasonable time. Such materials should be eliminated from aseptic installations and isolator systems decontaminated by VPHP. Smooth surfaces with brushed or polished finish are shown to have good inactivation behaviour. The results of these studies contribute to the improvement of VPHP decontamination processes in practice. They can be utilized for system optimization by operators of VPHP decontaminated systems as well as by manufacturers of isolators and gas generators.

## 6. Zusammenfassung

In der pharmazeutischen Industrie müssen sterile Medikamente, die nicht terminal sterilisierbar sind, unter aseptischen Bedingungen gefertigt und gehandhabt werden. Traditionell wurde die aseptische Medikamentenherstellung in Reinräumen mit hoher Raumklassifikation durchgeführt. Um die Produktsicherheit zu verbessern, ist die Anwendung von Isolatorsystemen, die den Verarbeitungsprozess mittels physikalischer Barrieren von der Umgebung und dem Bediener trennen, eine weitverbreitete Alternative. Bevor ein aseptischer Prozess durchgeführt werden kann, müssen die inneren Oberflächen des Isolators mit einem chemischen Sterilisationsmittel behandelt werden, um die mikrobielle Kontamination auf ein definiertes akzeptables Niveau zu reduzieren. Heute wird für diesen Zweck am häufigsten verdampftes Wasserstoffperoxid verwendet (Vaporized Hydrogen Peroxide, VPHP). Ein typischer Dekontaminationszyklus mit VPHP besteht aus den Phasen Vorbereitung, Konditionierung, Biodekontamination und Spülen. Unterschiedliche Zyklusparameter wie die Wasserstoffperoxidkonzentration, Feuchtigkeit und Kondensation haben einen Einfluss auf die mikrobizide Aktivität des Dekontaminationszyklus. Darüber hinaus beeinflussen auch Designfaktoren des Isolators wie das Konstruktionsmaterial und der geometrische Aufbau der Begasungskammer die Inaktivierungsergebnisse.

Obwohl heutzutage die Dekontamination mit verdampftem Wasserstoffperoxid in der pharmazeutischen und zugehörigen Industrien weitverbreitet ist, gibt es bisher in der Literatur noch kein einheitliches Verständnis hinsichtlich des Wirkmechanismus und optimaler Zyklusparameter. Die Zielsetzung der vorliegenden Dissertation war es, den Wirkmechanismus des verdampften Wasserstoffperoxids im Detail zu untersuchen, um eine Empfehlung für optimale Dekontaminationsbedingungen zu entwickeln. Darüber hinaus wurde angestrebt, den Einfluss von unterschiedlichen Konstruktionsmaterialien und Oberflächengüten sowie von geometrischen Strukturen auf die Inaktivierungsleistung des Dekontaminationsmittels hin zu untersuchen. Damit sollte das Design von aseptischen Produktionsmaschinen für die Dekontamination mit VPHP optimiert werden. Der Arbeit ist eine detaillierte Literaturübersicht zum aktuellen Stand des Wissens hinsichtlich der Dekontamination mit verdampftem Wasserstoffperoxid vorangestellt.

Für die Untersuchungen wurde ein pharmazeutischer 8 m<sup>3</sup> Testisolator mit angeschlossenem VPHP-Generator verwendet. Es wurden standardisierte Dekontaminationszyklen mit verschiedenen Peroxid- und Wasserkonzentrationen sowie Zykluszeiten entwickelt. Bioindikatoren (BIs) mit definierten Ausgangspopulationen von *Geobacillus stearothermophilus* Sporen wurden während der verschiedenen Dekontaminationszyklen exponiert. Diese Testorganismen sind bekannt für ihre besonders hohe Resistenz gegenüber verdampftem Wasserstoffperoxid und werden standardmäßig für die Validierung von VPHP-Zyklen in der pharmazeutischen Industrie eingesetzt. Über die Bestimmung von Inaktivierungskinetiken für die Test-Mikroorganismen konnte die sporizide Wirksamkeit jeder Kombination von Zyklusbedingungen ermittelt werden. Die verwendeten mikrobiologischen Methoden waren die Quantifizierung von überlebenden Sporen nach definierten Expositionsintervallen mittels „Wahrscheinlichster Keimzahl“ („Most Probable Number“ MPN) sowie die Bestimmung von dezimalen Reduktionszeiten (D-Werte). Die Bioindikatoren wurden im Isolator nicht nur offen exponiert getestet, sondern auch exponiert innerhalb von Spalten. Dabei sollte das diffusive Eindringverhalten des verdampften Wasserstoffperoxids in kleine Öffnungen untersucht werden. Verschiedene Konstruktionsmaterialien wurden mit definierten Ausgangspopulationen von Sporen beimpft, um das Resistenzverhalten der Mikroorganismen auf unterschiedlichen Oberflächen zu prüfen.

Zusätzlich wurden die physikalisch-chemischen Eigenschaften der jeweiligen Materialien im Detail untersucht, um Rückschlüsse auf eine Korrelation von Oberflächencharakteristika und dem Resistenzverhalten der Sporen zu ermitteln.

Die Ergebnisse zeigen, dass mehrere Zyklusparameter die sporizide Aktivität des verdampften Wasserstoffperoxids beeinflussen. Die Ergebnisse zeigen weiterhin, dass der entscheidende Faktor für eine erfolgreiche Dekontamination die mikroskopische Wechselwirkung des Sterilisationsmittels mit den Keimen auf der Oberfläche ist. Da jedoch diese Wechselwirkung schwer quantifizierbar ist, muss der wichtigste makroskopische Parameter, die  $H_2O_2$ -Konzentration, fokussiert werden. Die ermittelten Ergebnisse und das vorgestellte Modell integrieren und erklären alle bisher veröffentlichten Publikationen, die bisweilen widersprüchlich zu sein schienen. Es wird aufgezeigt, dass die Mikrokondensation im nicht-sichtbaren Bereich entscheidend ist für eine zufriedenstellende Inaktivierungsleistung und dass darüber hinausgehende Kondensation im sichtbaren Bereich keine weitere Verbesserung der mikrobiziden Aktivität mit sich bringt. Die Daten veranschaulichen, dass unter der Voraussetzung, dass alle anderen Parameter konstant bleiben, die mikrobielle Inaktivierung mit steigender Wasserstoffperoxidkonzentration beschleunigt wird. Eine  $H_2O_2$ -Konzentration von 800 ppm stellt eine ausreichende Deposition von Sterilisationsmittel auf den Oberflächen sicher und resultiert in exzellenter und reproduzierbarer Abtötung. Für Konzentrationen des Sterilisationsmittels, die über 800 ppm hinausgehen, kann keine weitere Verbesserung der Inaktivierung beobachtet werden. Es wird gezeigt, dass für offen exponierte Bioindikatoren eine niedrigere Wasserstoffperoxidkonzentration (400 ppm) durch einen höheren Feuchtigkeitslevel ausgeglichen werden kann. Der erhöhte Wassergehalt in der Dekontaminations-Atmosphäre begünstigt den Niederschlag des Dekontaminationsmittels. Je höher die Wasserstoffperoxidkonzentration ist, desto unabhängiger wird der Inaktivierungserfolg vom Feuchtigkeitsgehalt der Atmosphäre. Für Peroxidkonzentrationen im Bereich von 800 ppm ist die mikrobizide Wirksamkeit unabhängig von der Wasserkonzentration.

Im Gegensatz zu offen exponierten Bioindikatoren kann eine mangelnde  $H_2O_2$ -Konzentration bei Indikatoren, die innerhalb von Spalten exponiert sind, nicht durch eine höhere Feuchtigkeit kompensiert werden. Innerhalb der Spalten können ausschließlich die Höhe der Wasserstoffperoxidkonzentration sowie die Dauer des Zyklus den Dekontaminationserfolg beeinflussen. Es wird gezeigt, dass prinzipiell auch komplexe Strukturen mit VPHP dekontaminiert werden können, das Eindringverhalten jedoch limitiert ist. Mit sinkendem Spaltquerschnitt und mit zunehmender Tiefe der Spalten wird die Inaktivierung zunehmend erschwert. Wenn möglich, sollen solche verdeckten Oberflächen und schmale Spalten bei der Herstellung von aseptischen Produktionssystemen vermieden werden. Wenn dies nicht möglich ist, müssen bestimmte Designkriterien berücksichtigt werden (Spalthöhe > 4 mm, Spalttiefe < 30 mm).

Es wird demonstriert, dass die Daten, die mit dem Spaltmodell generiert wurden, auf reale Bauteile übertragbar sind. Es wird dargelegt, dass verschiedene Konstruktionsmaterialien und Oberflächenbeschaffenheiten das Resistenzverhalten von Sporen gegenüber VPHP beeinflussen. Es werden Materialien mit guter und schlechter Dekontaminationscharakteristik identifiziert und potentielle Ursachen für das ungleiche Verhalten ermittelt. Es wird veranschaulicht, dass rauhe Oberflächen, insbesondere solche mit stark poröser Struktur, die Sporen teilweise vor dem Sterilisationsmittel schützen, so dass eine reproduzierbare Dekontamination mit vertretbaren Zykluszeiten nicht gewährleistet werden kann. Solche Materialien sollten aus VPHP dekontaminierten aseptischen Installationen und Isolatoren entfernt werden. Es wird dargestellt, dass glatte Oberflächen mit gebürsteter oder polierter

## Zusammenfassung

Ausführung ein gutes Inaktivierungsverhalten zeigen. Die Ergebnisse der Arbeit tragen dazu bei, VPHP-Prozesse in der Praxis zu verbessern. Sie können sowohl von VPHP-Anwendern in der pharmazeutischen Industrie als auch von Herstellern von Isolatoren und Gasgeneratoren genutzt werden, um die entsprechenden Systeme zu optimieren.

## 7. Curriculum Vitae

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## **8. Erklärung**

Hiermit erkläre ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst und lediglich unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Insbesondere erkläre ich, dass ich nicht früher oder gleichzeitig einen Antrag auf Eröffnung des Promotionsverfahrens unter Vorlage der hier eingereichten Dissertation gestellt habe.

Stuttgart, im März 2010

Beatriz Unger-Bimczok