Bio-decontamination with Hydrogen Peroxide (H_2O_2): Fundamentals

PDA Parenteral Drug Association

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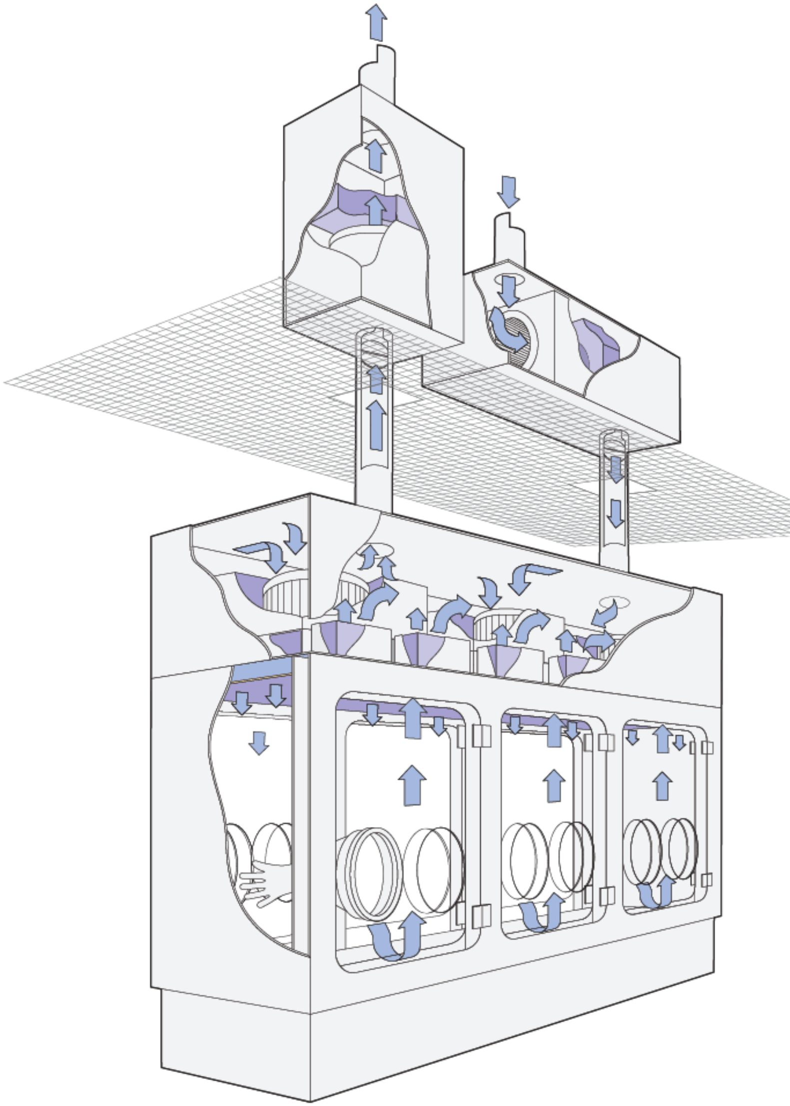
Isolator technology

- Separation of the process and operators
- Aseptic processing ~ handling of a product while preventing its (microbial) contamination

- Key Functions
  - Maintenance of Aseptic state
    - HEPA filtration
    - Unidirectional airflow
    - Differential pressure (gradient)
    - Transfer systems
    - Physical separation (gloves)
  - Establishment of aseptic state
    - (Cleaning / Disinfection)
    - Bio-decontamination
    - (Sterilization)



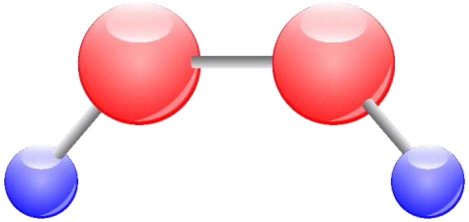
Bio-decontamination

- Process that reduces viable bioburden to acceptable level via use of sporicidal chemical agents

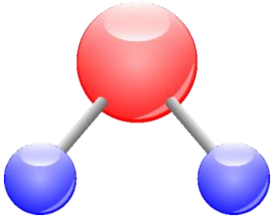
Key applications
- Bioburden management: room bio-decontamination, material transfer airlocks/hatches
- Preparation of an isolator for aseptic processing (production)

Hydrogen peroxide (H_2O_2)

- Why do we use H_2O_2 ?
  - Broad non-specific activity against microorganisms
  - Low toxicity, safe to use
  - Active at low temperatures and ambient pressure
  - Good material compatibility
  - Acceptable storage stability
  - Environmentally green solution
- Why vapor form ?
  - Complex, yet highly effective
  - Vapor may be efficiently distributed over the enclosure
  - It allows automated “No touch” process that can be validated
  - Established technology
    -> over 25 years of successful history



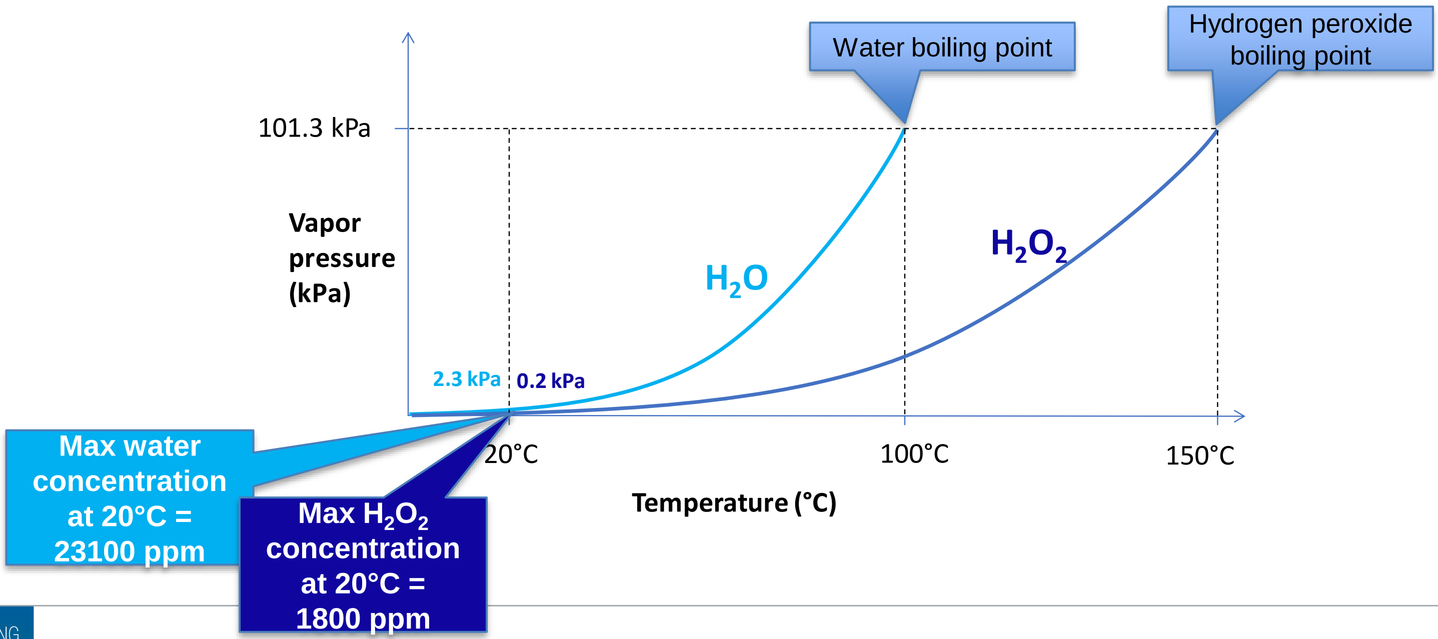
Hydrogen peroxide
BP: 150°C / 302°F



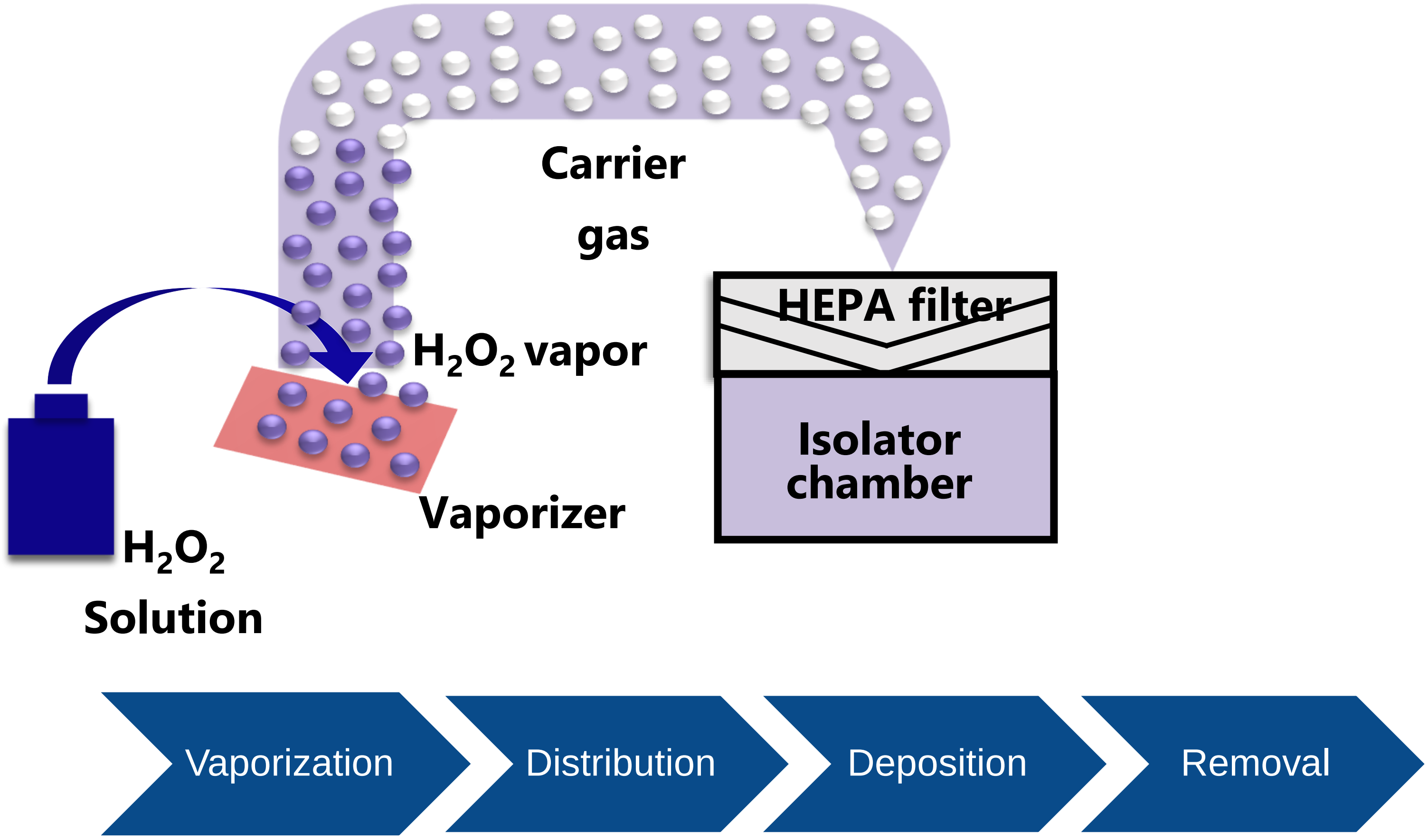
Water
BP: 100°C / 212 °F

Vapor

- Vapor refers to molecules in a gas phase of a substance that at given temperature exists as a liquid (or a solid)
- Each substance has a limit (maximal) vapor concentration depending on the temperature "Saturation vapor pressure"
- H_2O_2 is less volatile than water (approx. 10x) -> evaporated H_2O_2 condenses preferably



Bio-decontamination basic principle



Bio-decontamination agents

- In the bio-decontaminated chamber, we find the following actors:
  - AIR molecules - always present as gas – 78% N_2, 21% O_2, 1% Ar, etc..
  - WATER molecules - present as gas (i.e. vapor) or as liquid (i.e. droplets, condensate)
  - H_2O_2 molecules - present as gas (i.e. vapor) or as liquid (i.e. droplets, condensate)

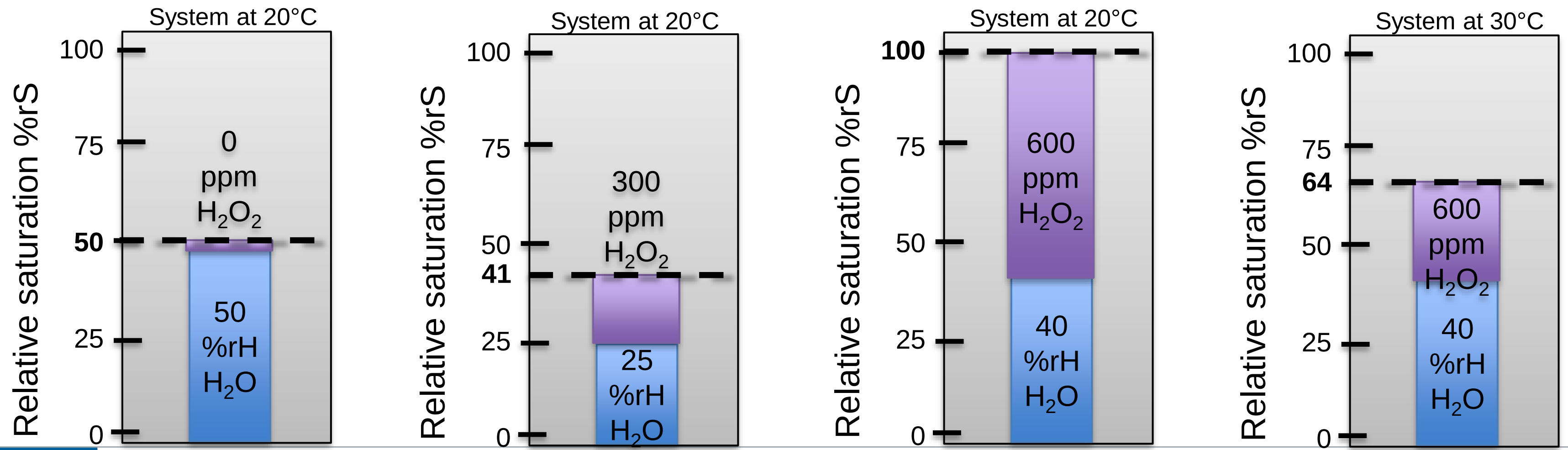
- H_2O_2 is the active agent responsible for the bio-decontamination effect thanks to its oxidative properties (especially the oxidative properties of induced radicals such as ·OH)
  - However, its presence and special distribution/concentrations are heavily influenced by water and temperature

- Water is an influencing agent, it swells proteins, induces H_2O_2 condensation, and influences oxidative radical reactions which are the key to inactivate microorganisms

- AIR acts as an inert
  - It may be used to accelerate H_2O_2 distribution by translational movements (active mixing)
  - It slows down the diffusion rate of H_2O_2/water molecules

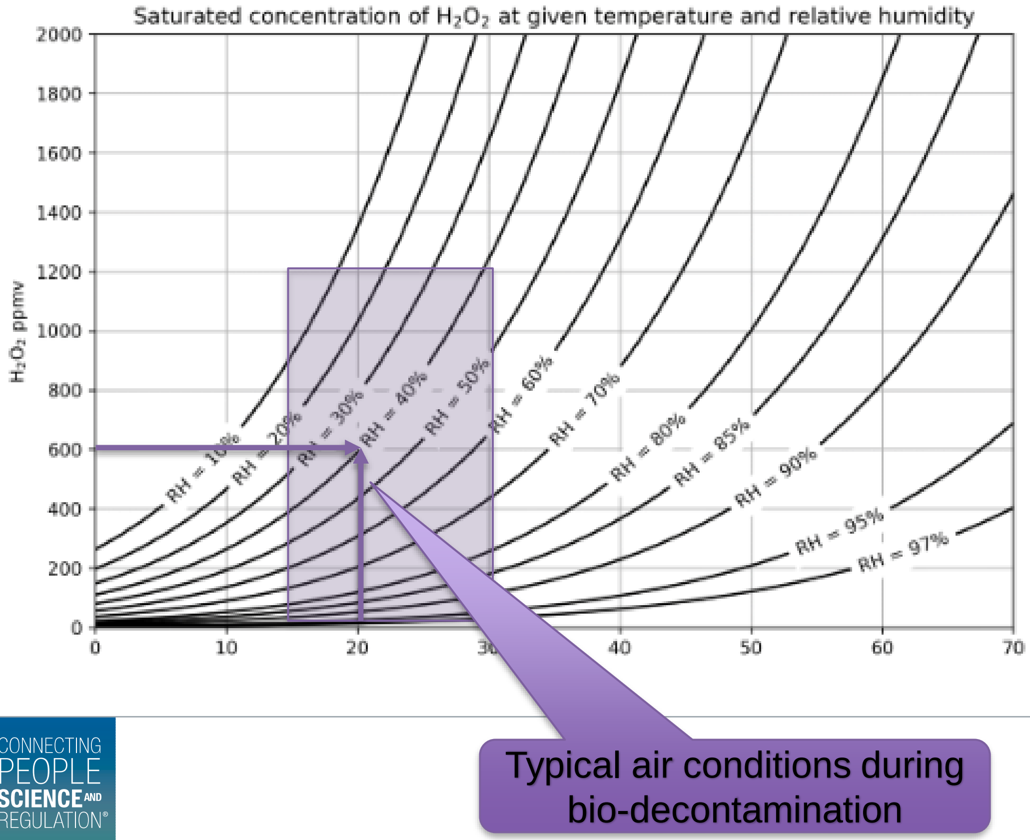
Relative Humidity and Saturation

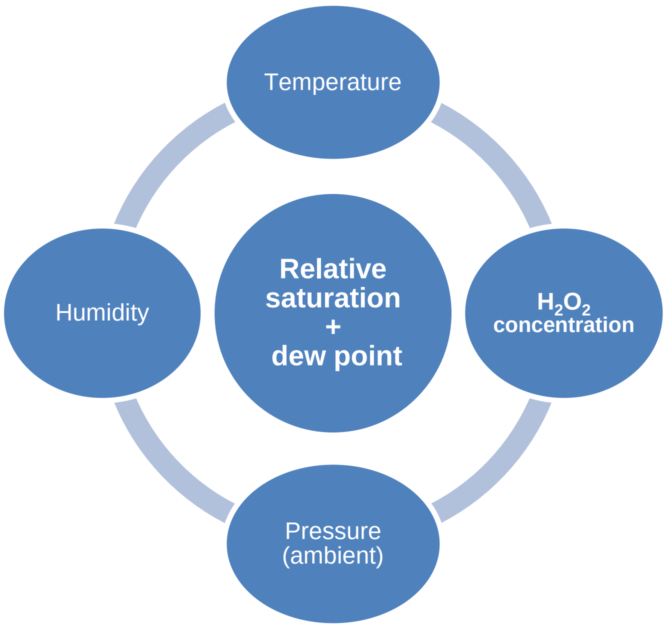
- Relative Humidity (%rH) represents the amount of water vapor in air
  - not so relevant for bio-decontamination
- Relative Saturation (%rS) represents the combined amount of water and H_2O_2 vapor in air
  - Relative saturation is used to express the remaining vapor capacity of air
  - In other words, it expressed the “willingness” of H_2O_2-water vapor to condense
- Higher relative humidity ↑ -> lower maximal H_2O_2 vapor concentration ↓
- Higher temperature ↑ -> higher maximal H_2O_2 vapor concentration ↑



Key bio-decontamination parameters

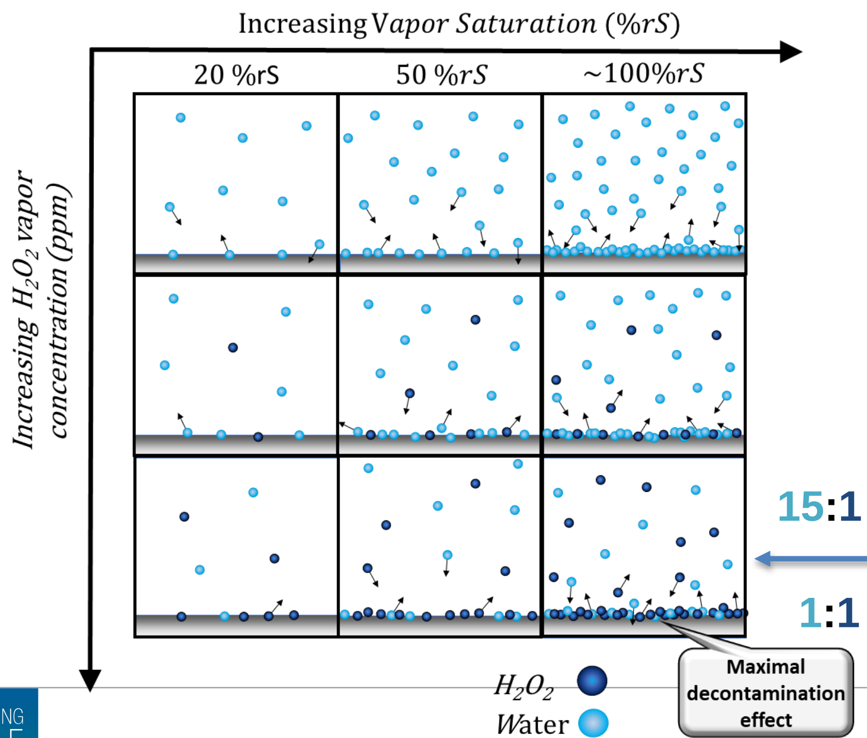
- Key parameters: CONTACT TIME, H_2O_2 vapor concentration and relative saturation
- Microbial inactivation rate increases (=> better bio-decontamination effect) with
  - Longer contact time, higher H_2O_2 vapor concentration, higher relative saturation

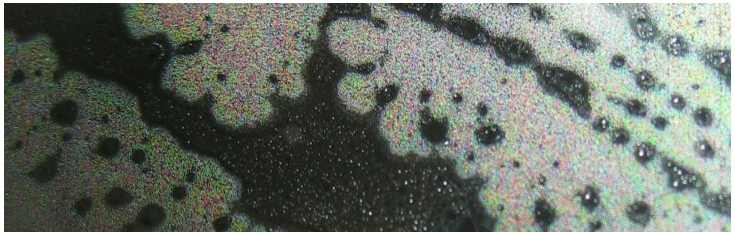




H_2O_2 deposition

- Adsorption appears on all surfaces in contact with hydrogen peroxide/water vapor
- The adsorbed layer thickness increases with increasing relative saturation
- Visible condensation appears on surfaces that are below the dew point temperature
- The concentration of H_2O_2 in adsorbate/condensate is much higher than in the vapor phase





Visible condensation

Example – vapor-liquid equilibrium
20°C, 600ppm H_2O_2, 40% rH (9000ppm water)
-> deposition of 60%wt H_2O_2

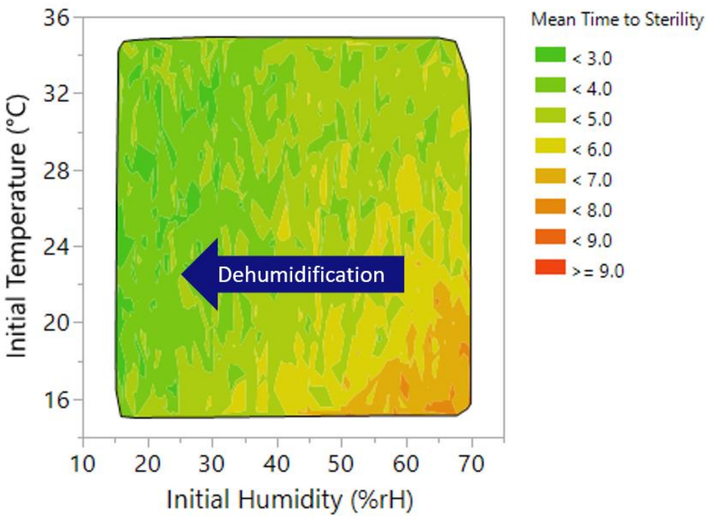
Environmental effects

- Decontamination is typically performed at ambient conditions
  - Humidity
  - Temperature
  - Pressure

- Higher humidity -> Less air capacity for H_2O_2 vapor -> Lower max efficacy
- Lower temperature -> Less air capacity for H_2O_2 vapor -> Lower max efficacy
- Pressure influence insignificant

- WORST CASE -> low temperature + high humidity

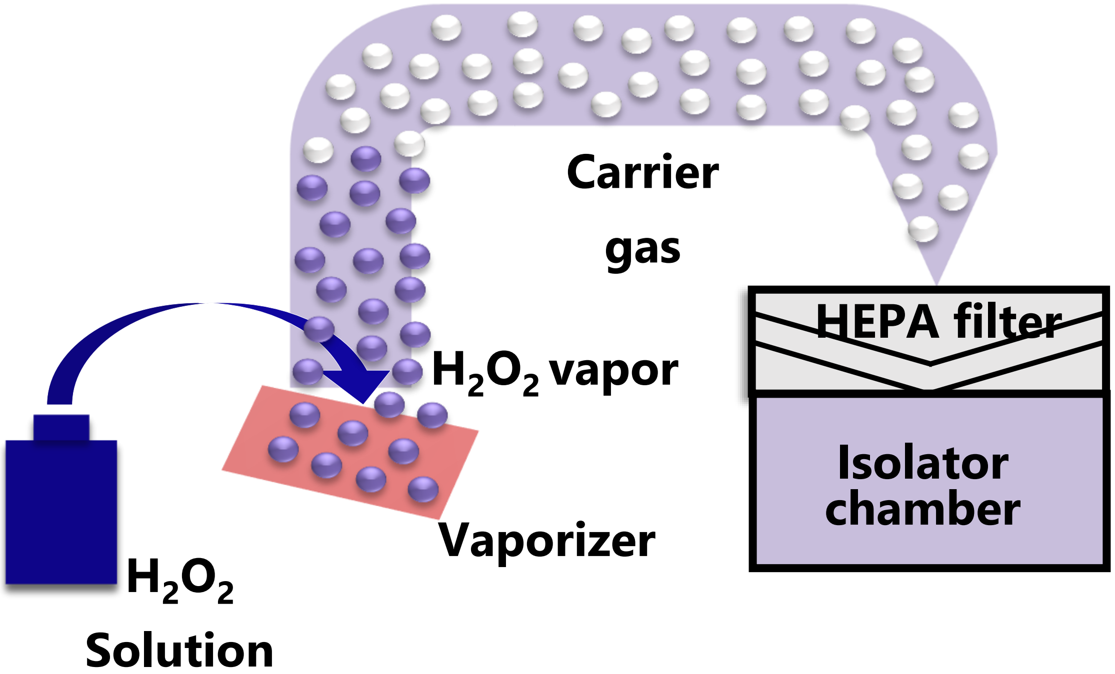
- Dehumidification applied to eliminate process variations due to humidity fluctuations



Effect of temperature locally

- Deposition of H_2O_2 on a surface decreases with increasing surface temperature
- Importance of temperature mapping for cycle development

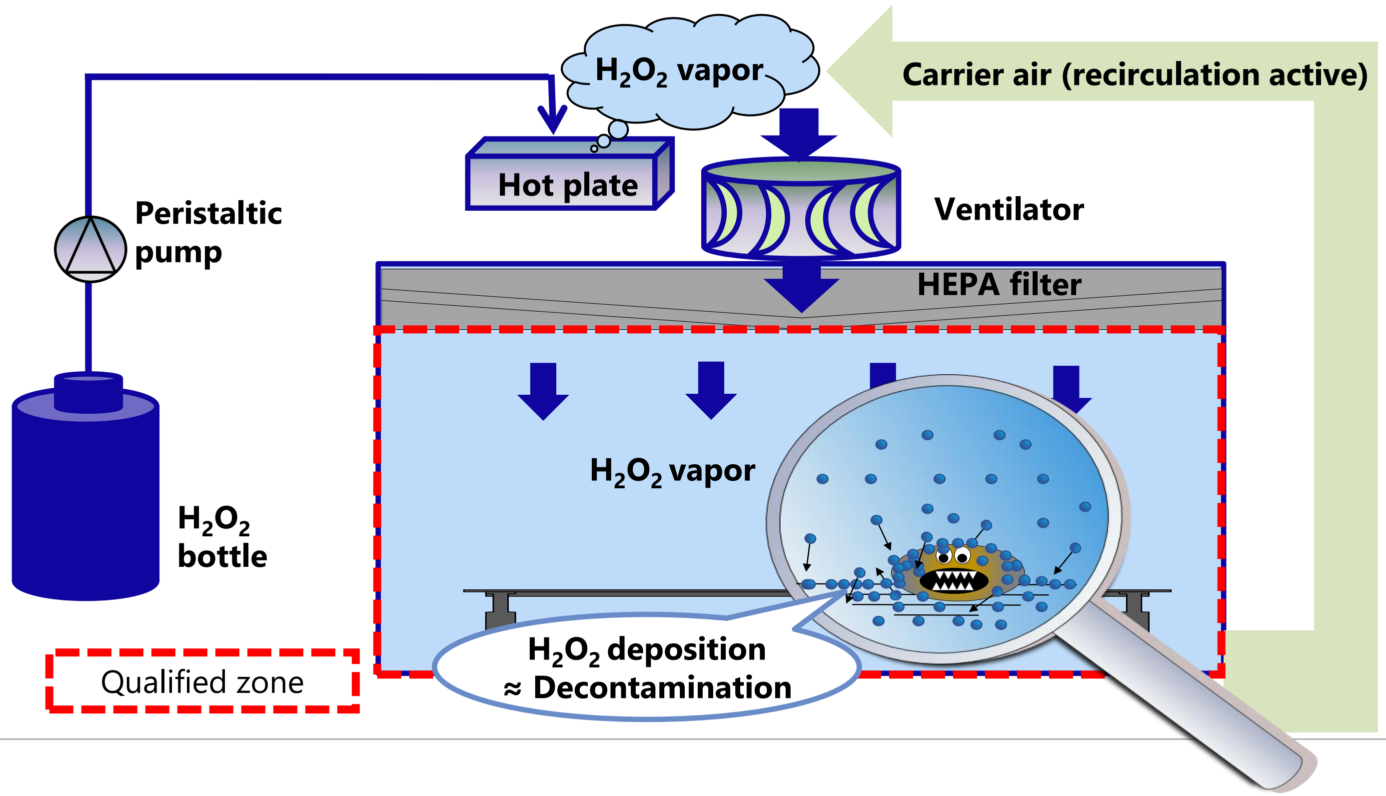
Basic principle



Vaporization → Distribution → Deposition → Removal

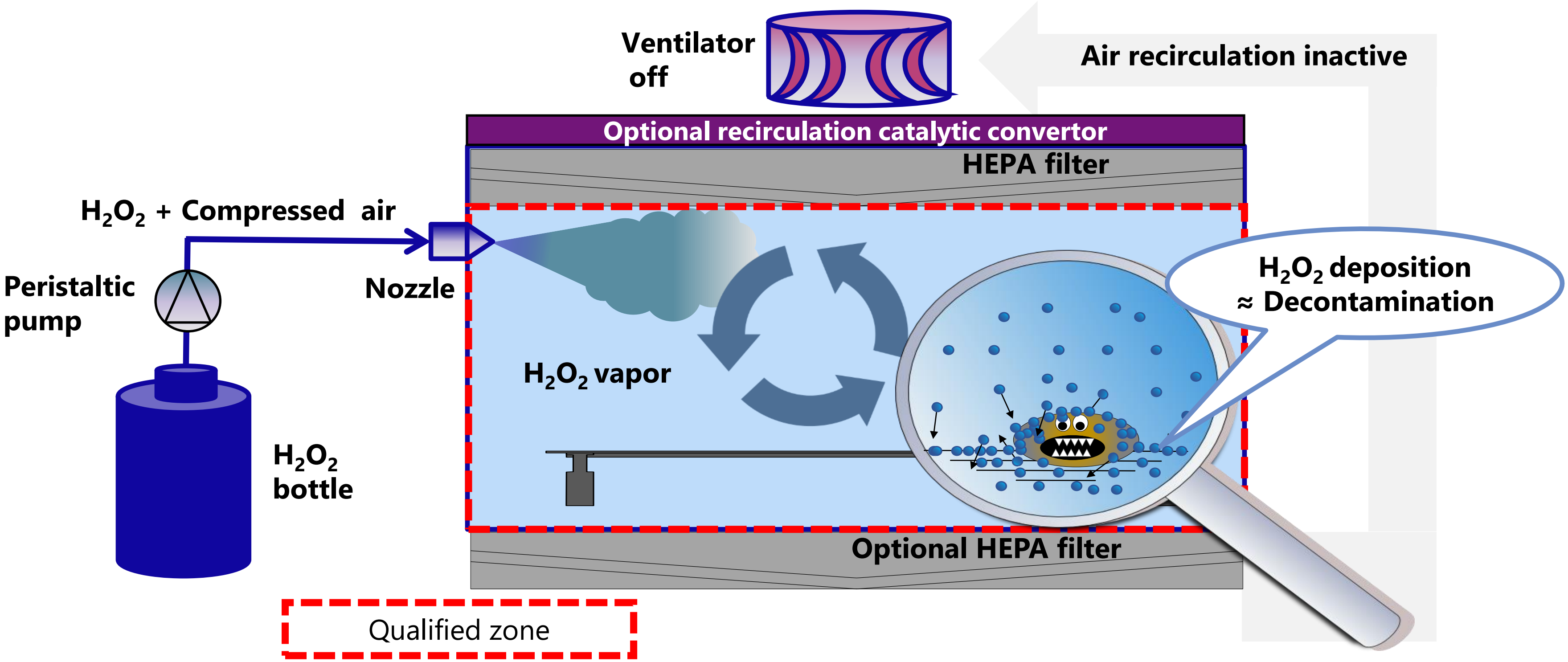
Hot plate evaporation

Example – SIS-700 System



Evaporation by fogging

Example – skanfog - micro-nebulization



Fogging vs Hot plate

Fogging

- Robust and effective
- "Cold" vaporization
- Allows fast H_2O_2 injection
- Less H_2O_2 consumed
- Reduced HEPA filter exposure
- Nozzle positioning flexible
- Flexible and scalable
- Cycle times <1 hour possible

Hot plate

- Robust and effective
- "Hot" vaporization
- Slower H_2O_2 injection required
- Higher H_2O_2 consumption
- Full HEPA filter exposure
- Fixed vaporizer positioning
- Less flexibility/scalability
- Cycle times <2 hours possible

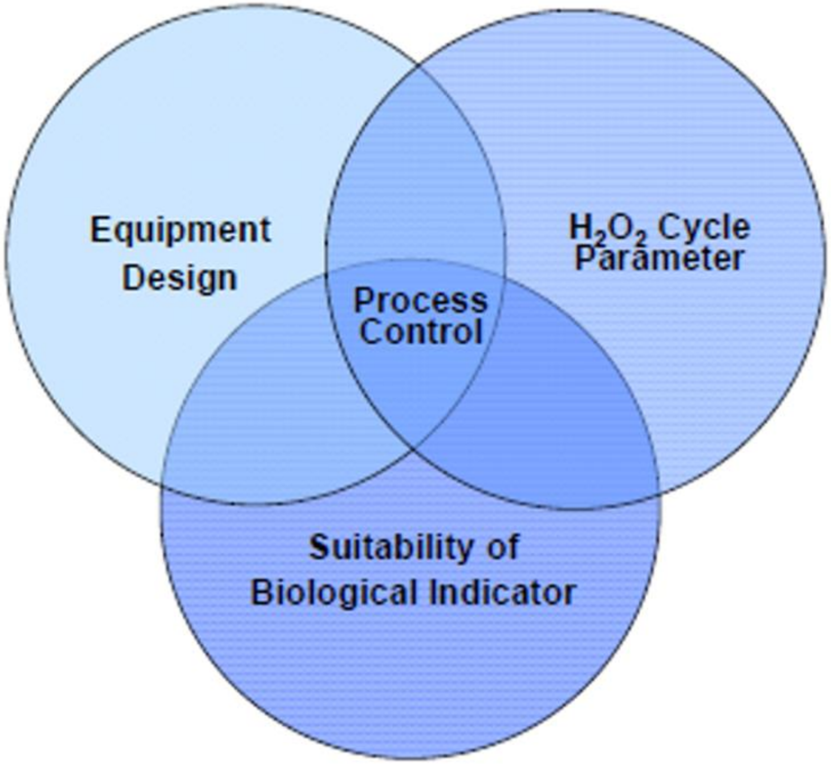
While the technology of vapor delivery is different, fundamentals remain the same!

Both technologies may offer benefits depending on the process needs

"VPHP", "aHP", "VHP", "HPV", etc..

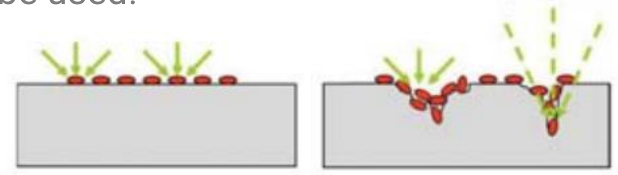
Process control

- The same general principles apply for all H_2O_2 vapor phase bio-decontamination techniques
- Key Factors:
  - Equipment design
  - Justification of cycle parameters during cycle development
  - Suitable Biological indicator and other tools
  - Process expectations, QRM (deco effect, residual H_2O_2)



Equipment design

- Only materials suitable for H_2O_2 bio-decontamination should be used!
  - Material properties (e.g. porosity, affinity to water)
  - Material persistence (e.g. chemical resistance)
  - H_2O_2 absorptivity
  - Catalytic activity
  - TESTING, not assuming

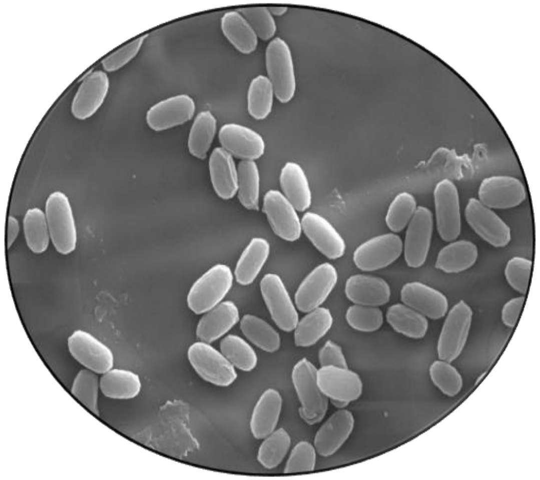


- Hygienic design and system accessibility to assure good cleanability of the surfaces
- Good H_2O_2 distribution (no “dead end” cavities, minimize weak spots, active homogenization)
- Criticality of loading pattern (cycle development)
- Keep temperature variation within acceptable level (cycle development)

Biological indicators

- Tools for evaluation of microbial inactivation processes
- BI consists of homogeneously distributed biocontamination on a metal carrier packed in permeable membrane

- BIs developed for H_2O_2 decontamination
  - Spores of *Geobacillus Stearothermophilus (highly resistant to H_2O_2 processes)*
  - BIs with excess of 10^4, 10^5 or 10^6 CFU/carrier
  - Carrier material - Stainless steel
  - Primary packaging - Tyvek®
  - Custom BIs can also be used

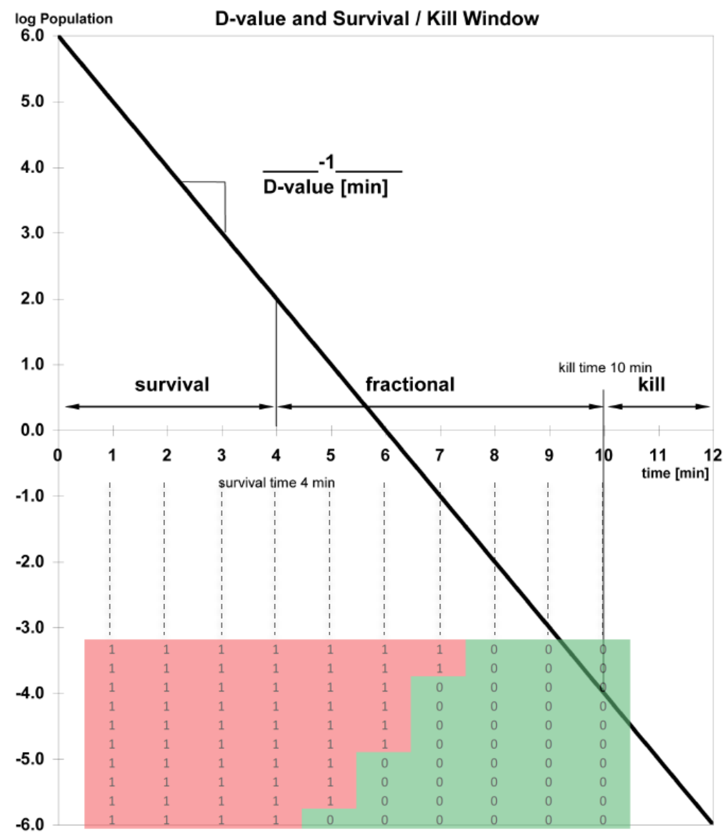


"BI is a characterized preparation of a specific microorganism that provides a defined and stable resistance to a specific microbial inactivation process" (*USP* <55>)

"The biological indicator provides a means to directly assess the sterilizing effect of the method in a manner not possible by physical measurements." (USP<1229>)

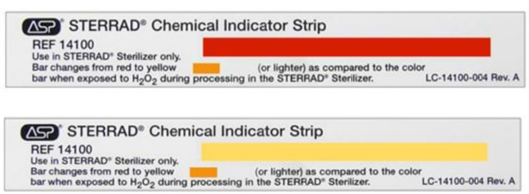
BI resistance

- Resistance of BIs is typically expressed as D-value
- D-value is defined as a time needed to reduce viable population on the BI carrier by 90% (i.e. 1 log reduction) when exposed to bio-decontamination "kill" conditions
- For H_2O_2 standard "kill" conditions do NOT exist
- Resistances given by BI manufacturers in CoAs are informative only -> do not consider labeled D-value as your system D-value
- Methods differ significantly among vendors! request information about the method
- Importance of model behavior – within lot variability Lot should behave homogeneously, minimum of late positives



Chemical indicators (CIs)

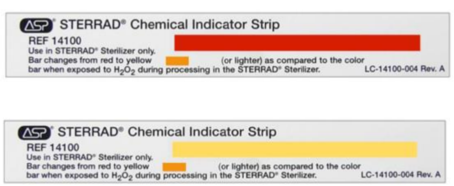
- Qualitative CIs play minimal (yet sometimes very useful) role
  - Immediate and simple readout (color change visible with naked eye)
  - Qualitative indication of H_2O_2 presence only
  - Weak information with regards to cycle effectiveness
  - Quick check of the decontamination homogeneity/ distribution
  - Can be used for troubleshooting, design optimization purposes



Enzyme Indicators – emerging quantitative CIs

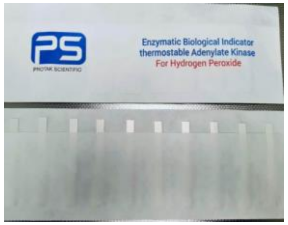
- Enzyme Indicators (EIs) allow quantitative readout after the cycle
- Highest price and effort required among other CIs
- **More information / data can be collected with EIs, but BI's remain the only proof**
  - What does the EI data mean?
  - Is the effort of collecting the data worth it?
  - Hybrid strategies (BIs + EIs) are being investigated over the industry

Chemical indicator evolution



Qualitative CIs

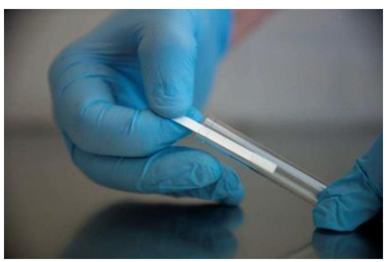
Semi-Quantitative CIs

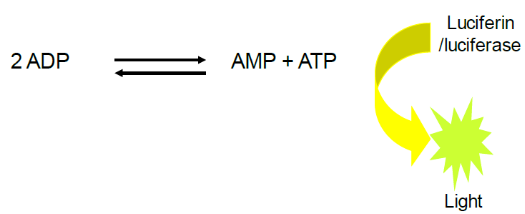


Quantitative CIs

Enzyme indicator technology principle

- **Sensing principle:** Degradation of thermostable enzyme by H_2O_2
- **Readout principle:** Light generated during chemical reaction catalyzed by the enzyme
- **Quantification:** Light measurement inside of a luminometer -> RLU (relative light units)
- **Interpretation:** Less Light generated = More enzyme degradation = More H_2O_2 (effect)





Considerable effort and cost

Inactivation rates of EIs and BIs may not change in synchrony

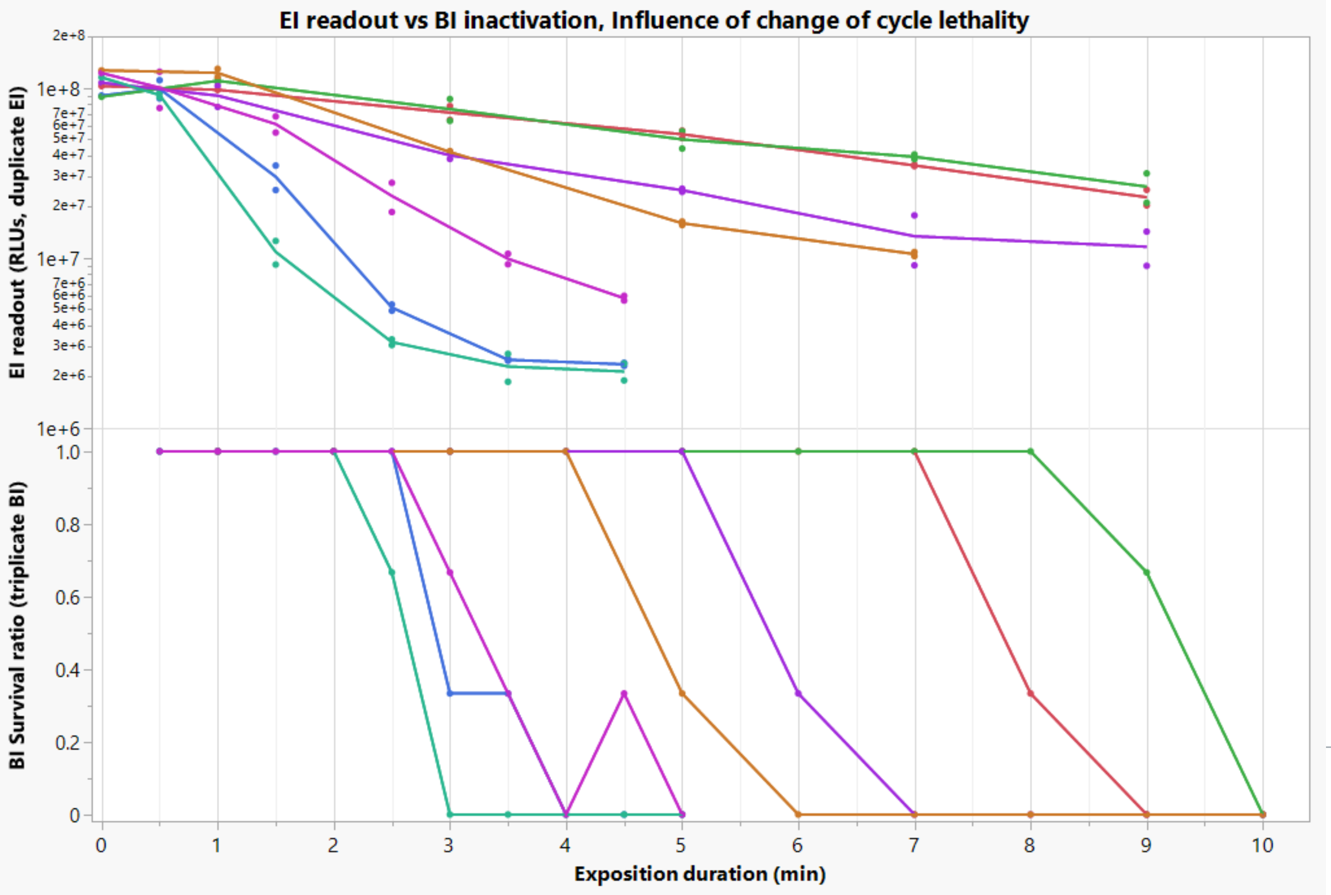
- **Example:** 1 technology, 1 BI lot, 1 system and sample location, variation of cycle lethality

- Changes of cycle lethality result in change of EI and BI inactivation rate

faster BI kill = faster EI inactivation

- EI readout (RLU value) corresponding to BI kill time changes with cycle lethality

1 Equation relating RLUs to log reduction is not applicable



Sensors: measurement of key in-process parameters

- Temperature
- Humidity
- H_2O_2 concentration (High and Low)
- (Relative saturation / Dew point)

- There is no harmonized model relating key in-process parameters and H_2O_2 decontamination effect (i.e. BI kill / spore log reduction)

- Trending of in-process parameters allows for very good indication of cycle reproducibility -> **Cycle Health**

Bio-decontamination expectations

- Integrated and automated process capable to reach all inner surfaces
- Proven robust effectiveness
  - Process must be validated + safety margin for robustness
  - Validation is performed with suitable Biological Indicators (BIs)
  - Total kill of 6 log BIs is typically expected
- Safe for operator and no impact on the processed product
  - After decontamination, the active agent concentration needs to be reduced to required safe level

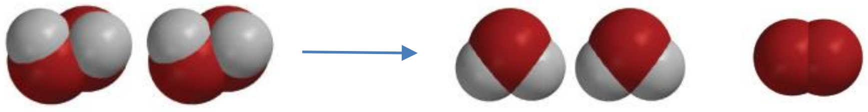
Hydrogen peroxide (delivered in vapor form) is the most common agent in the industry

Residual H_2O_2 target

- Definition of Target H_2O_2 level
  - Typical target is <1ppm (or <0.5ppm) considering operator safety
  - Products may be extremely sensitive to oxidation and thus lower concentrations of 0.1ppm or even lower towards 30ppb are sometimes needed
  - Use spiking studies and trace H_2O_2 exposure tests to determine right H_2O_2 aeration target with regards to product quality

- Optimization of aeration duration
  - Technology selection, novel airflow concepts and catalysts enable extra short cycle times
  - Wrong selection of loading material may ruin any short cycle goal
  - Preliminary testing of H_2O_2 ingress into various materials will prevent any possible issues
  - Each plastic material is different!

H_2O_2 catalysts



$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

H_2O_2 decomposes to harmless water (H_2O) and Oxygen (O_2)

- Degradation of H_2O_2 down to operator safe levels in a single pass through a catalyst
- Can greatly save time and energy requirements of the decontamination process.
- Terminal vs Recirculation catalysts
- Single-pass through catalysts able to degrade high levels of H_2O_2 are nowadays available

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Common misconceptions

- H_2O_2 decontamination is a gaseous process
  - NO, H_2O_2 decontamination is two phase liquid-vapor process
- Condensation must be prevented during the cycle
  - NO, quickly reaching saturation and micro-condensation on surfaces makes inactivation quicker (also the surfaces above the dew point temperature become bio-decontaminated, but it typically takes longer)
- Condensation will damage the materials
  - NO, only materials tested to be persistent to H_2O_2 should be used in isolators and therefore this is not a concern (may be a concern for room bio-decontamination)
- Cycles able to get a "total kill" of 6 log BI (8-9 log reduction) assure robust process
  - NO, H_2O_2 bio-decontamination has limited penetrability and therefore only suitable materials (e.g. non-porous) shall be used; surfaces need to be sufficiently clean prior to the cycle
- D-values on BI certificates will apply for any H_2O_2 decontamination system
  - NO, D-values will differ system to system, the certified D-value may be used only to estimate lot-to-lot differences of a specific BI product/type, not much more

Thank you for your attention!

Questions?

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