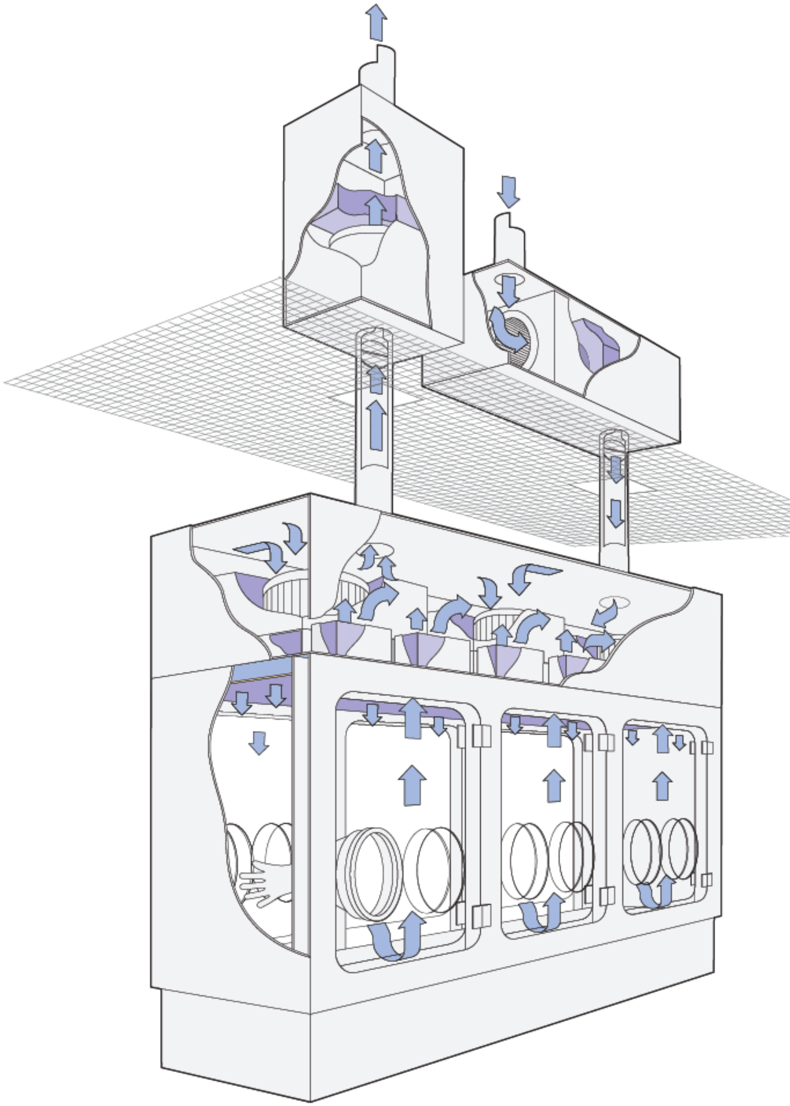
(bio)Decontamination with Hydrogen Peroxide (H_2O_2): Fundamentals

Isolator technology

- Separation of the process and operators
- Aseptic processing ~ handling of the 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)
    - Decontamination
    - (Sterilization)



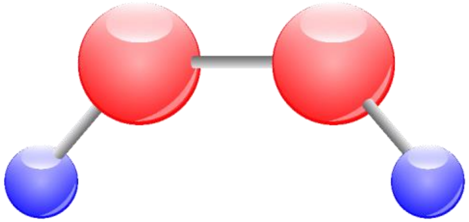
Decontamination

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

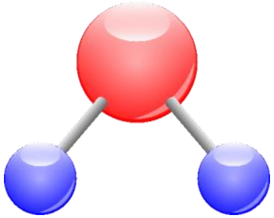
Key applications
- Bioburden management: room decontamination, material transfer airlocks/hatches
- Preparation of an isolator for aseptic processing (production)

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



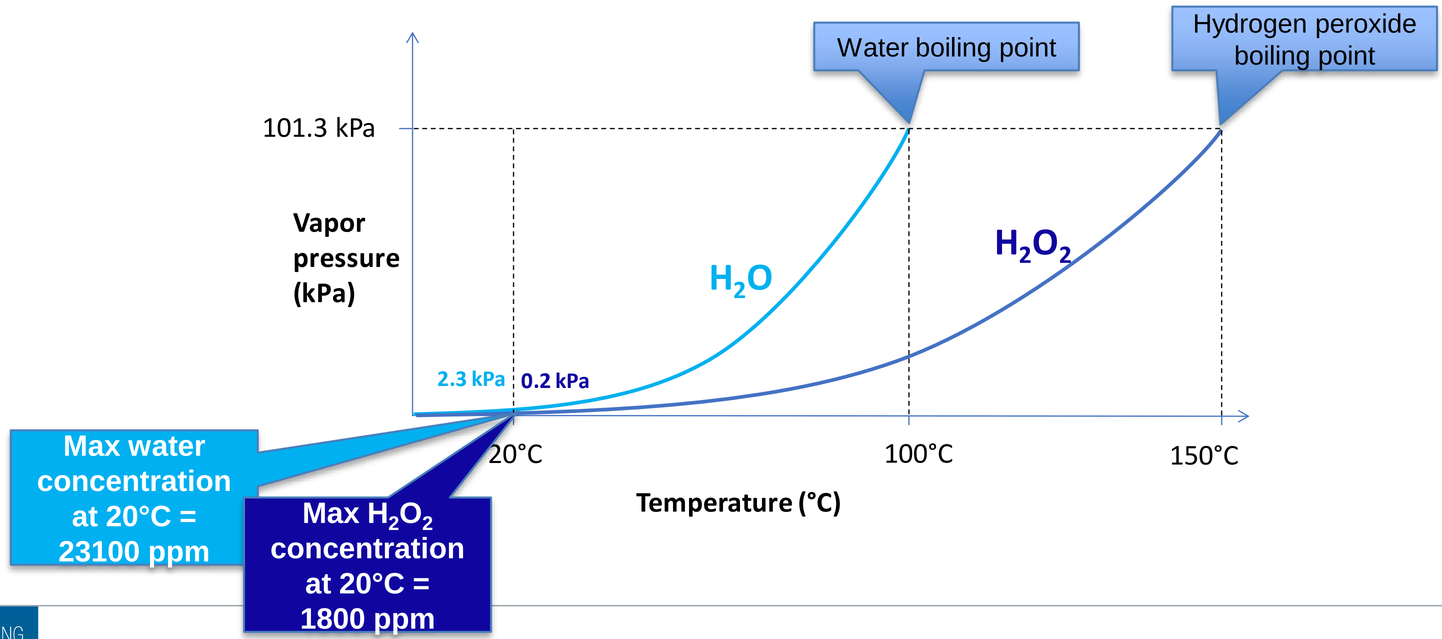
Hydrogen peroxide
BP: 150°C / 302°F



Water
BP: 100°C / 212 °F

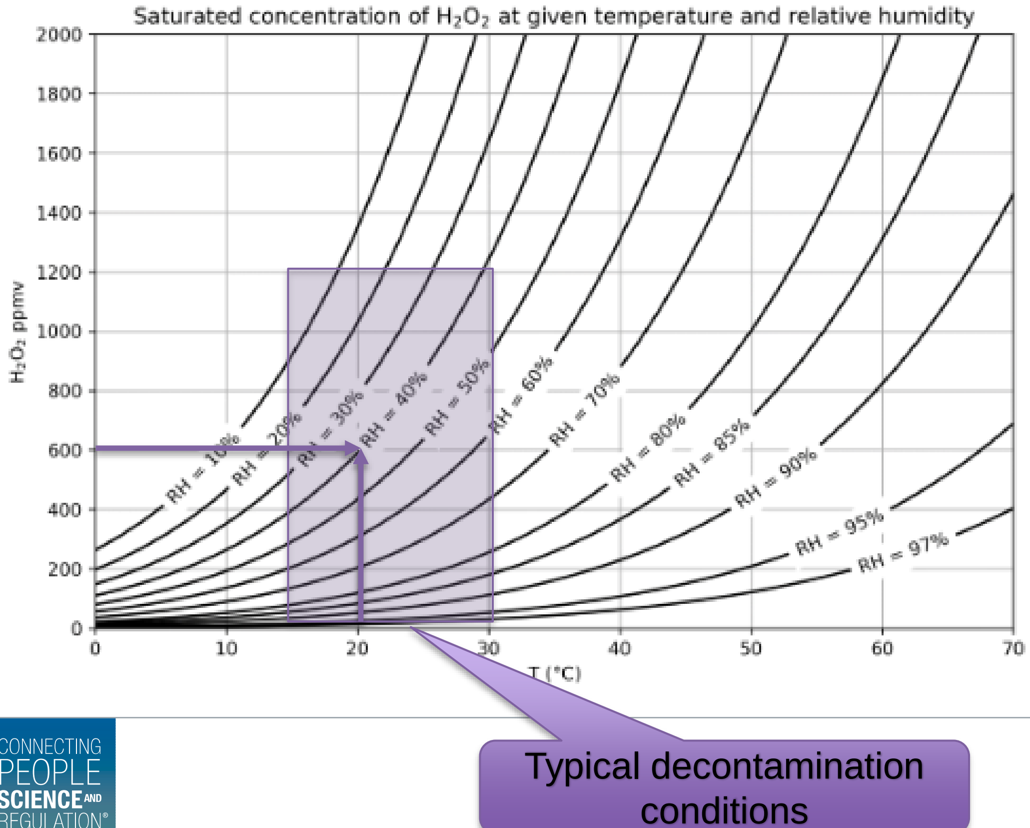
Vapor

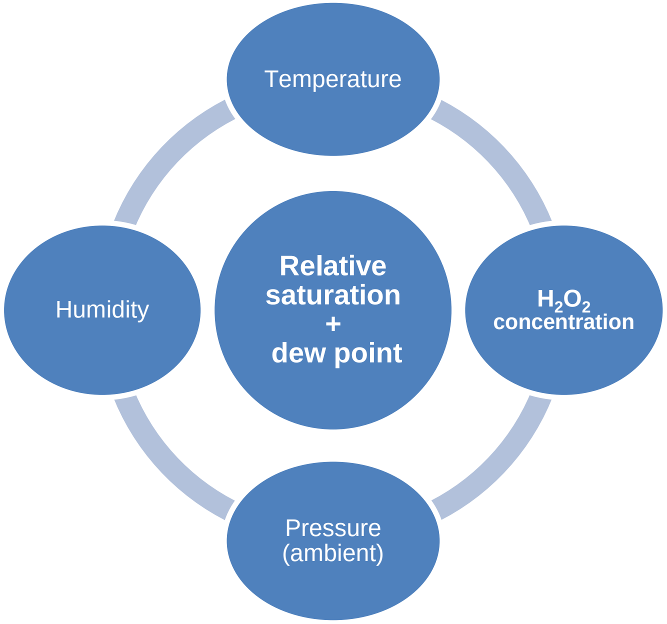
- Vapor refers to molecules in a gas phase of a substance that at given temperature exists as a liquid (or a solid)
- Each substance has a limit (maximal) vapor concentration depending on the temperature "Saturation vapor pressure"
- H_2O_2 is less volatile than water (approx. 10x) -> evaporated H_2O_2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



Key decontamination parameters

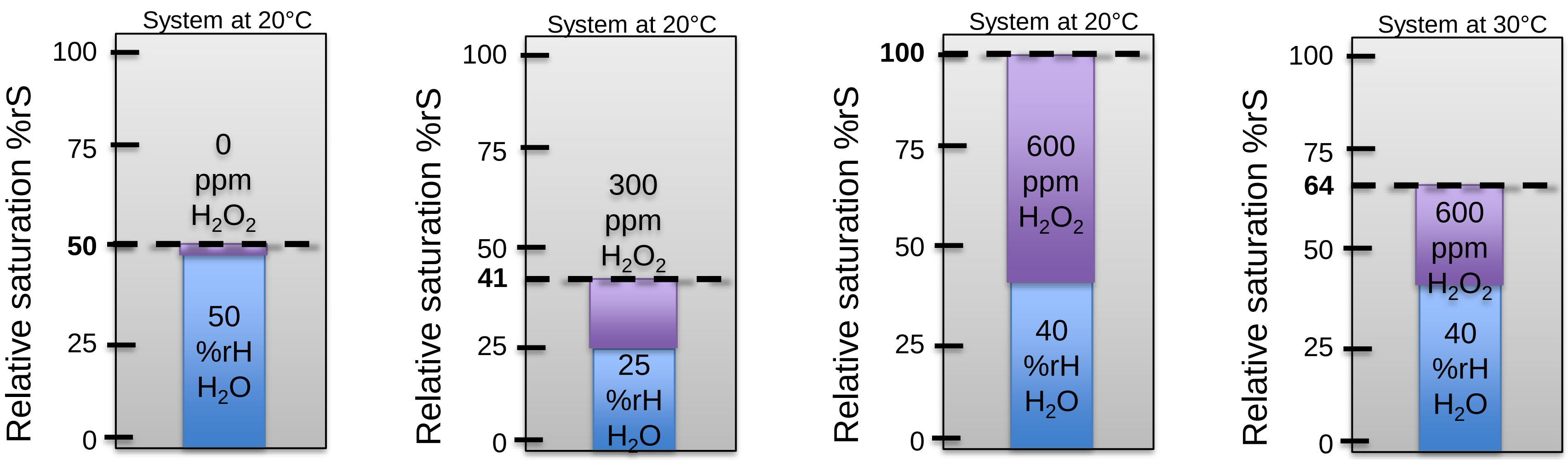
- Key parameters: CONTACT TIME, H_2O_2 vapor concentration and relative saturation
- Microbial inactivation rate increases (=better decontamination effect) with
  - Longer contact time, Higher H_2O_2 vapor concentration, higher relative saturation





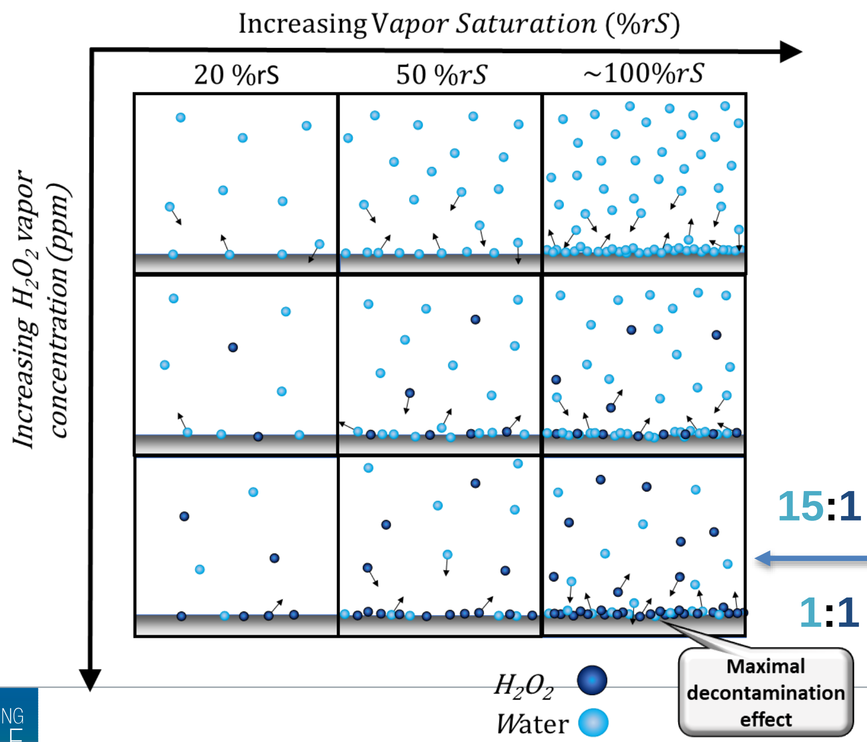
Relative Humidity and Saturation

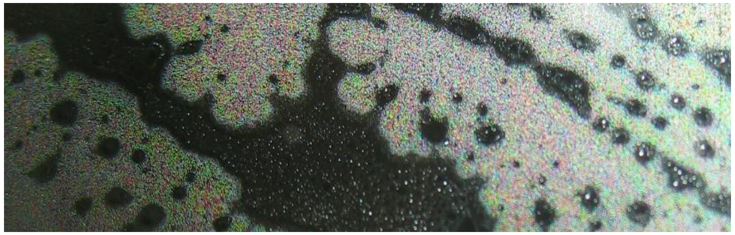
- Relative Humidity (%rH) represents the amount of water vapor in air
- Relative Saturation (%rS) represents the amount of water and H_2O_2 vapor in air
- With increasing humidity, maximal achievable H_2O_2 vapor concentration decreases
- With increasing temperature, higher H_2O_2 vapor concentration can be reached



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

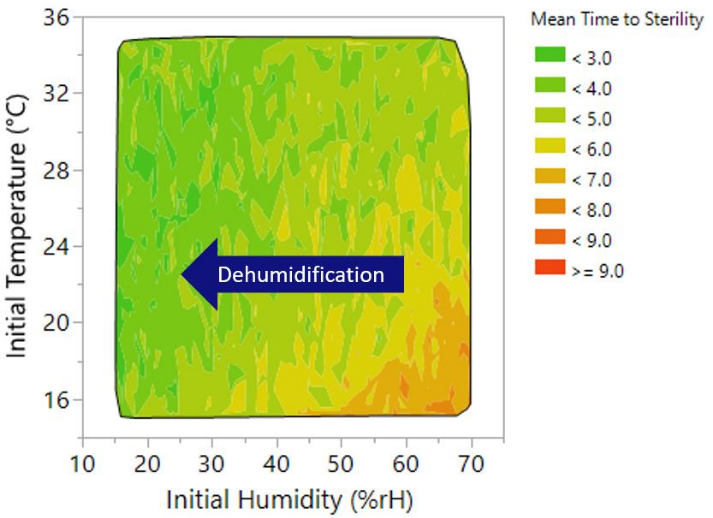
Environmental effects

- 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

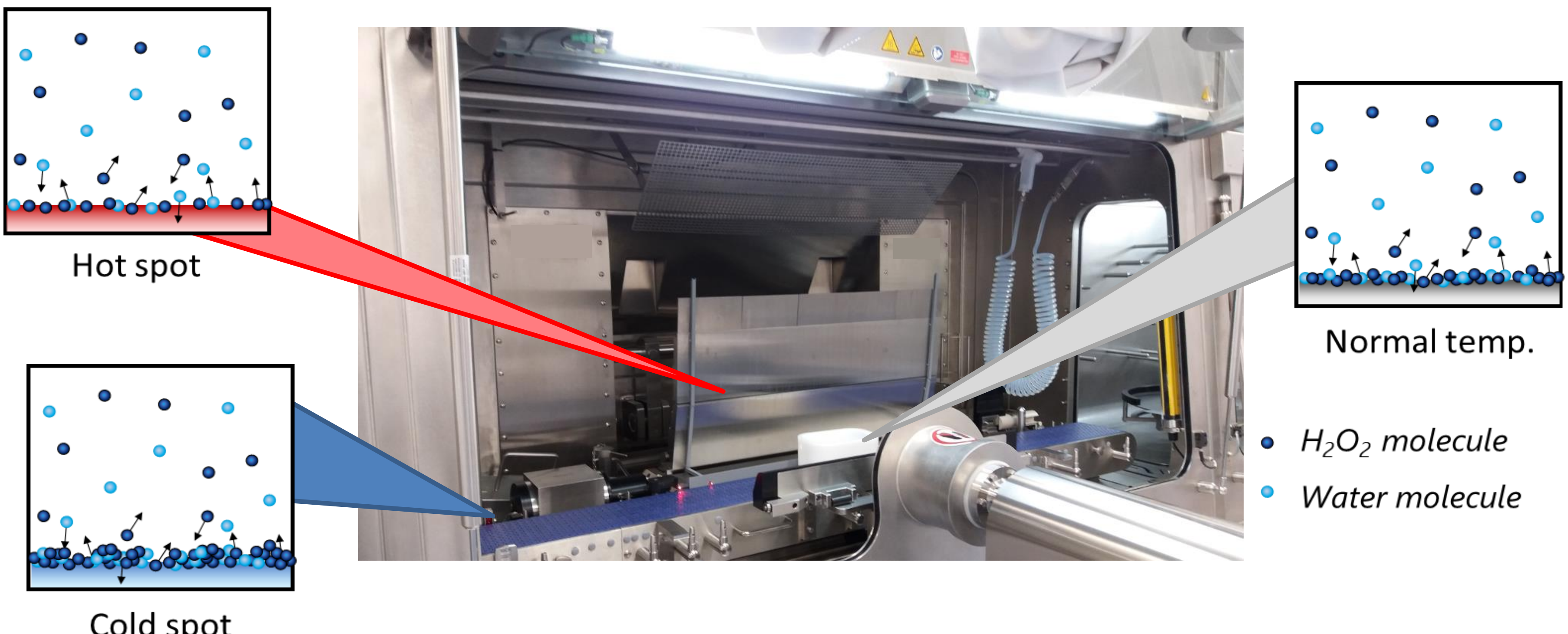
- WORST CASE -> low temperature + high humidity

- Dehumidification applied to eliminate process variations due to humidity fluctuations

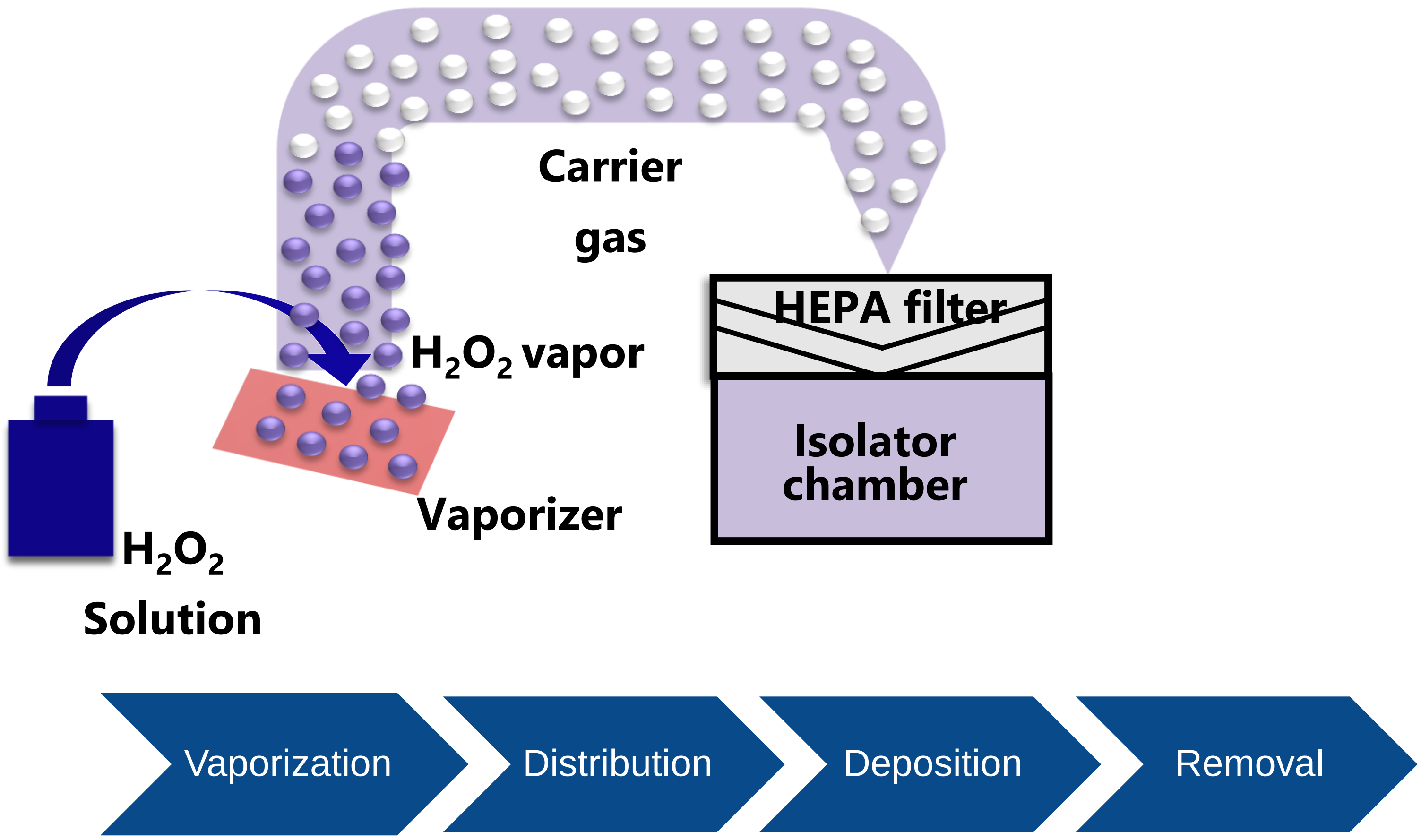


Effect of temperature locally

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

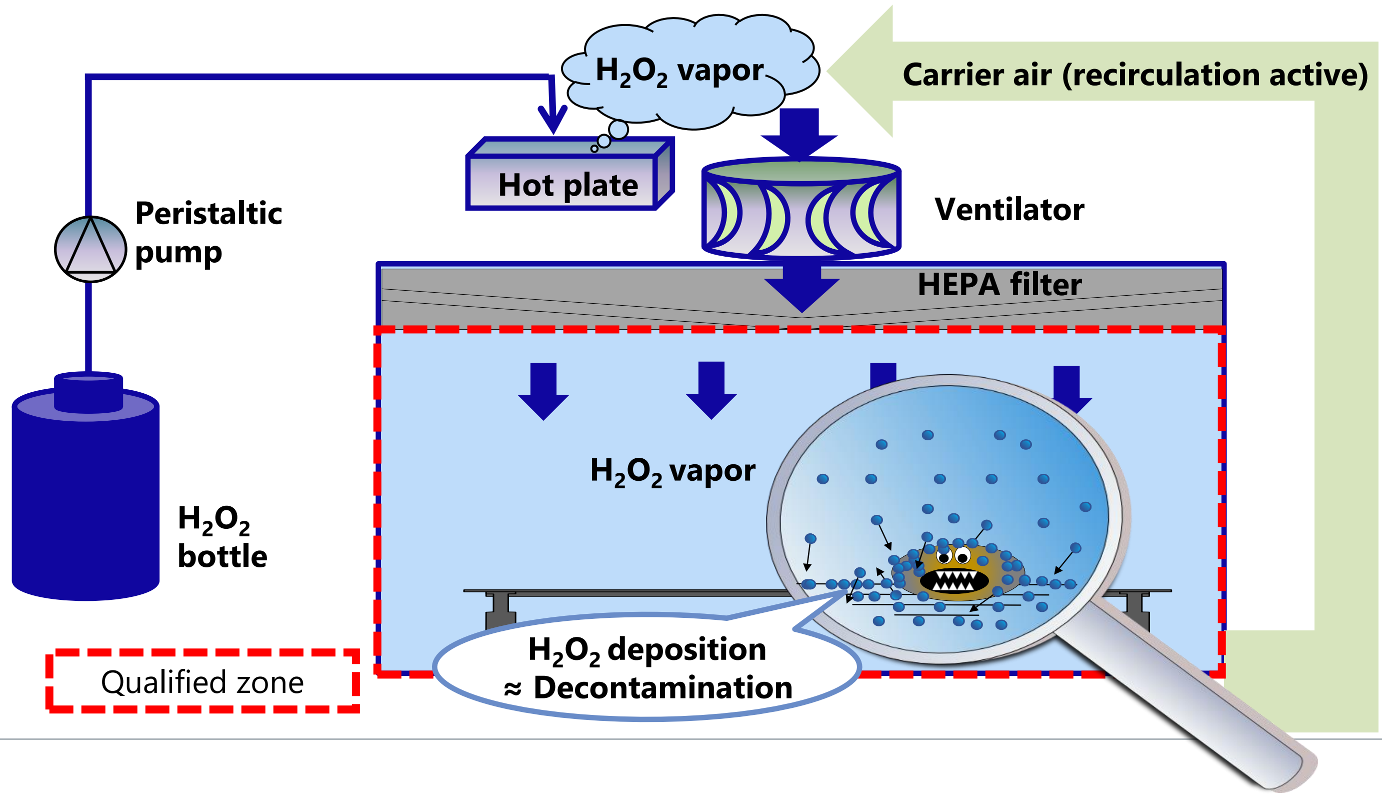


Basic principle



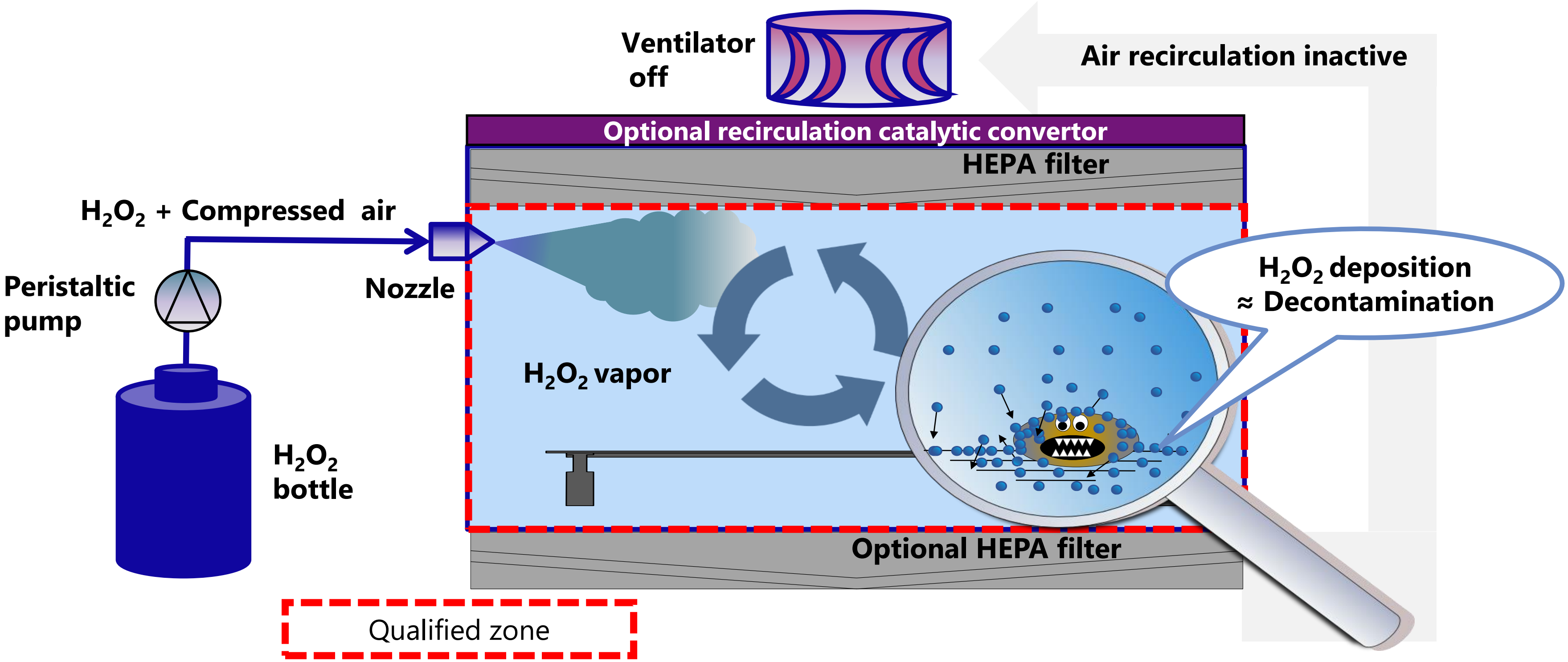
Hot plate evaporation

Example – SIS-700 System



Evaporation by fogging

Example - skanfog® micro-nebulization



Fogging vs Hot plate

- 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

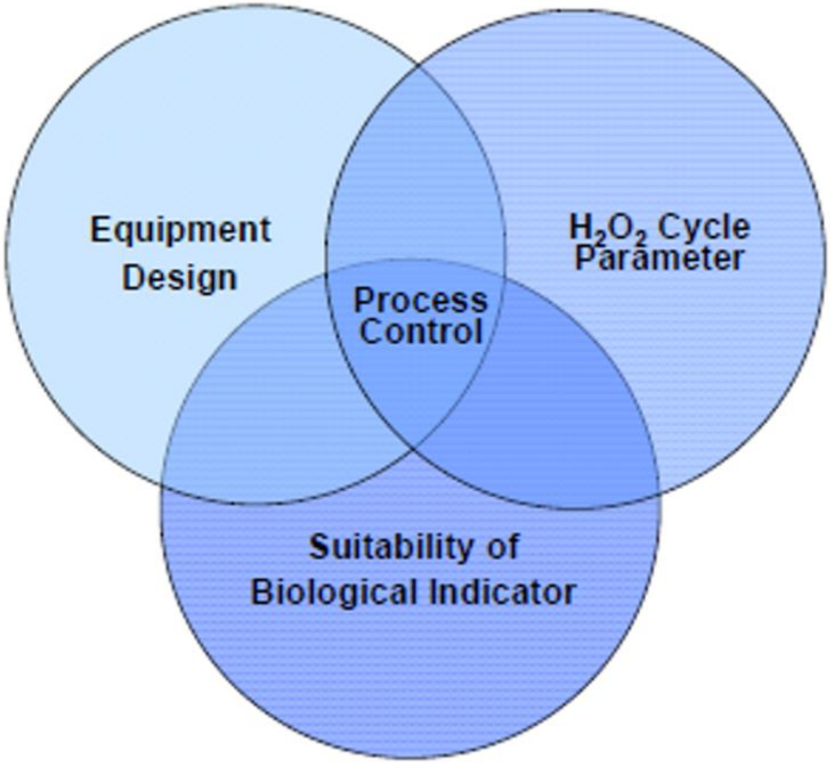
- Robust and effective
- “Hot” vaporization
- Slower H_2O_2 injection required
- Higher H_2O_2 consumption
- Full HEPA filter exposure
- Fixed vaporizer positioning
- Less flexibility/scalability
- Cycle times <2 hours possible

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

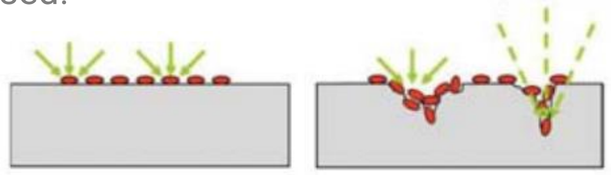
Both technologies may offer benefits depending on the process needs

Process control

- The same general principles apply for all H_2O_2 vapor phase 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

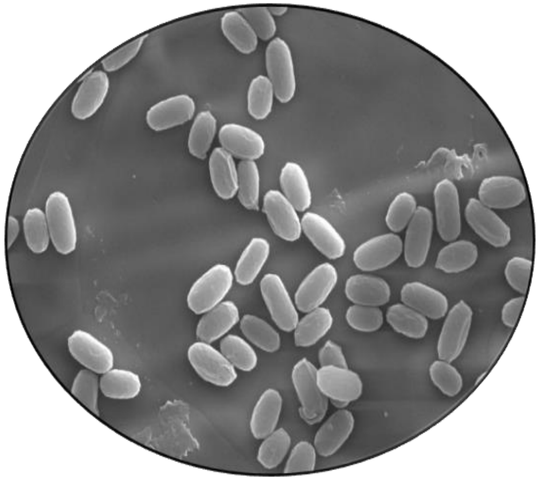


- Only materials suitable for H_2O_2 decontamination should be used!
  - Decontamination aspects (e.g. porosity)
  - Material persistence (e.g. chemical resistance)
  - H_2O_2 absorptivity
  - Catalytic activity
  - TESTING, not assuming
- Hygienic design and system accessibility to assure good cleanability 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

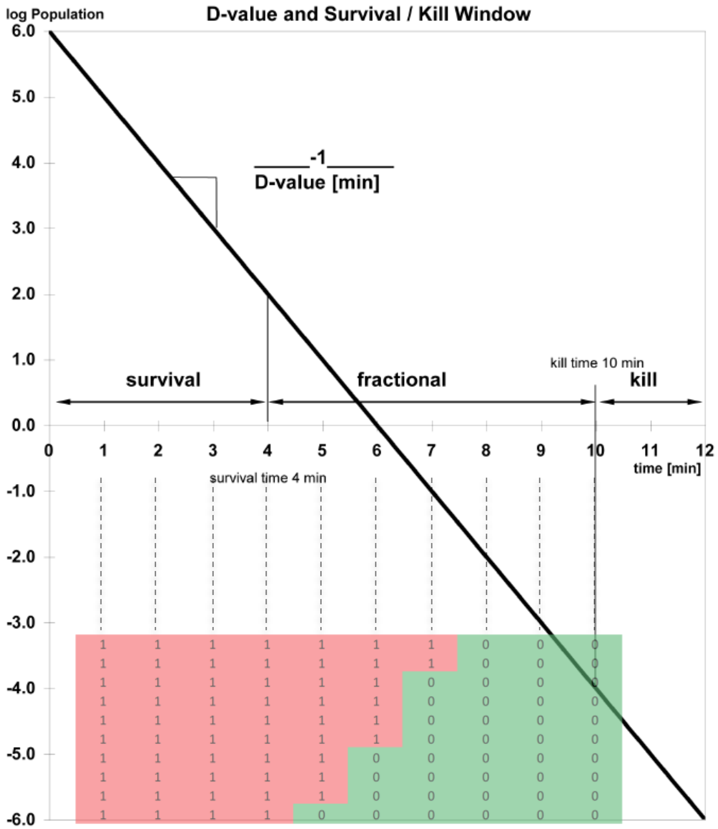


"BI is a characterized preparation of a specific microorganism that provides a defined and stable resistance to a specific microbial inactivation process" (*USP* <55>)

"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>)

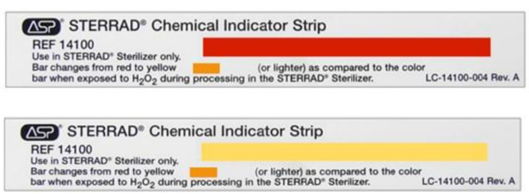
BI resistance

- 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)
- For H_2O_2 standard "kill" conditions are not defined
- Resistances given by BI manufacturers are informative only (e.g. lot-to-lot variability)
- 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



Sensors: inline 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**

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
  - Typically target of 1ppm (or 0.5ppm) is based on operator safety requirements
  - Products may be extremely sensitive to oxidation and thus lower concentrations of 0.1ppm or even lower towards 30ppm are sometimes needed
  - Perform spiking studies and trace H_2O_2 exposure tests to justify the H_2O_2 aeration target
- 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!

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 even condensation on surfaces makes inactivation quicker (also the surfaces above the dew point temperature become decontaminated, but it 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 decontamination)
- Inactivation of 6 log BI assures robust process
  - NO, H_2O_2 decontamination has limited penetrability and therefore only suitable materials (e.g. non-porous) shall be used; surfaces need to be 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 assess lot-to-lot differences of a specific BI type

Thank you for your attention!

Questions?

Martin Novák
Technology Lead
Martin.novak@skan.ch