Al-Farabi Kazakh National University

Sterilization Process

Lecture 5

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A fermentation product is produced by the culture of a certain organism, or animal cell line, in a nutrient medium.

Sterilization is the process to eliminate microorganisms from a medium or equipment.

It is used to:

- Pre Sterilized equipments
- Sterilization of feedstocks
- Maintenance of sterile operating conditions

If a foreign microorganism invades the fermentation then the following consequences may occur:

The medium would have to support the growth of both the production organism and the contaminant, resulting in a loss of productivity.

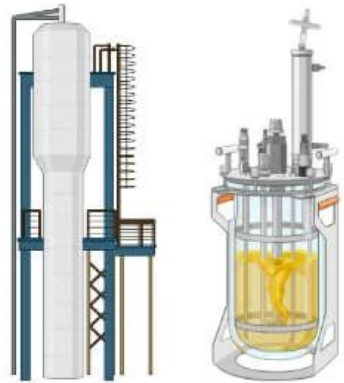
If the fermentation is a continuous one then the contaminant may “outgrow” the production organism and displace it from the fermentation.

The foreign organism may contaminate the final product

The contaminant may produce compounds that make subsequent extraction of the final product difficult.

The contaminant may degrade the desired product; this is common in bacterial contamination of antibiotic fermentations where the contaminant would have to be resistant to the normal inhibitory effects of the antibiotic and degradation of the antibiotic is a common resistance mechanism, for example, the degradation of β-lactam antibiotics by β-lactamase-producing bacteria.

Contamination of a bacterial fermentation with phage could result in the lysis of the culture.



https://microbenotes.com/fermentation/

How to avoid contamination?

- Effective design and construction of the fermentation plant.
- Using a pure inoculum to start the fermentation
- Sterilizing the medium to be employed.
- Sterilizing the fermenter vessel.
- Sterilizing all materials to be added to the fermentation during the process, for example, air, nutrient feeds, antifoams, and pH titrants.
- Maintaining aseptic conditions during the fermentation.
- Putting in place detailed operating procedures for sterilization, aseptic maintenance, and staff training.

Sterilization Agents

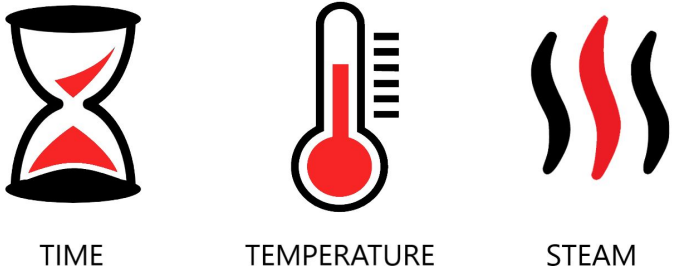
1.Thermal - preferred for economical large-scale sterilizations of liquids and equipment. Thermal sterilization uses the thermal lability of a microorganism to prevent its growth.

2. Chemical - preferred for heat-sensitive equipment

→ ethylene oxide (gas) for equipment

→ 70% ethanol-water (pH=2) for equipment/surfaces

→ 3% sodium hypochlorite for equipment

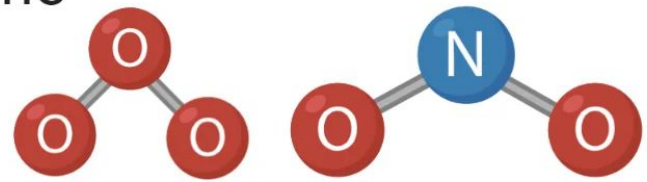


https://safesteril.etia-group.com/steam-sterilization/

Chemical Sterilization

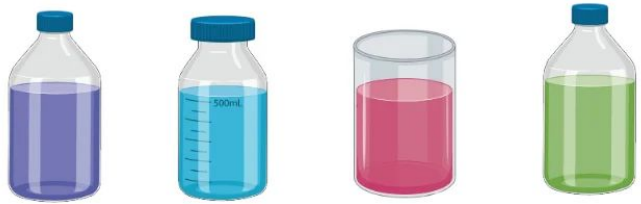
Gaseous Sterilization

- Ethylene oxide
- Formaldehyde
- Nitrogen dioxide (NO_2)
- Ozone



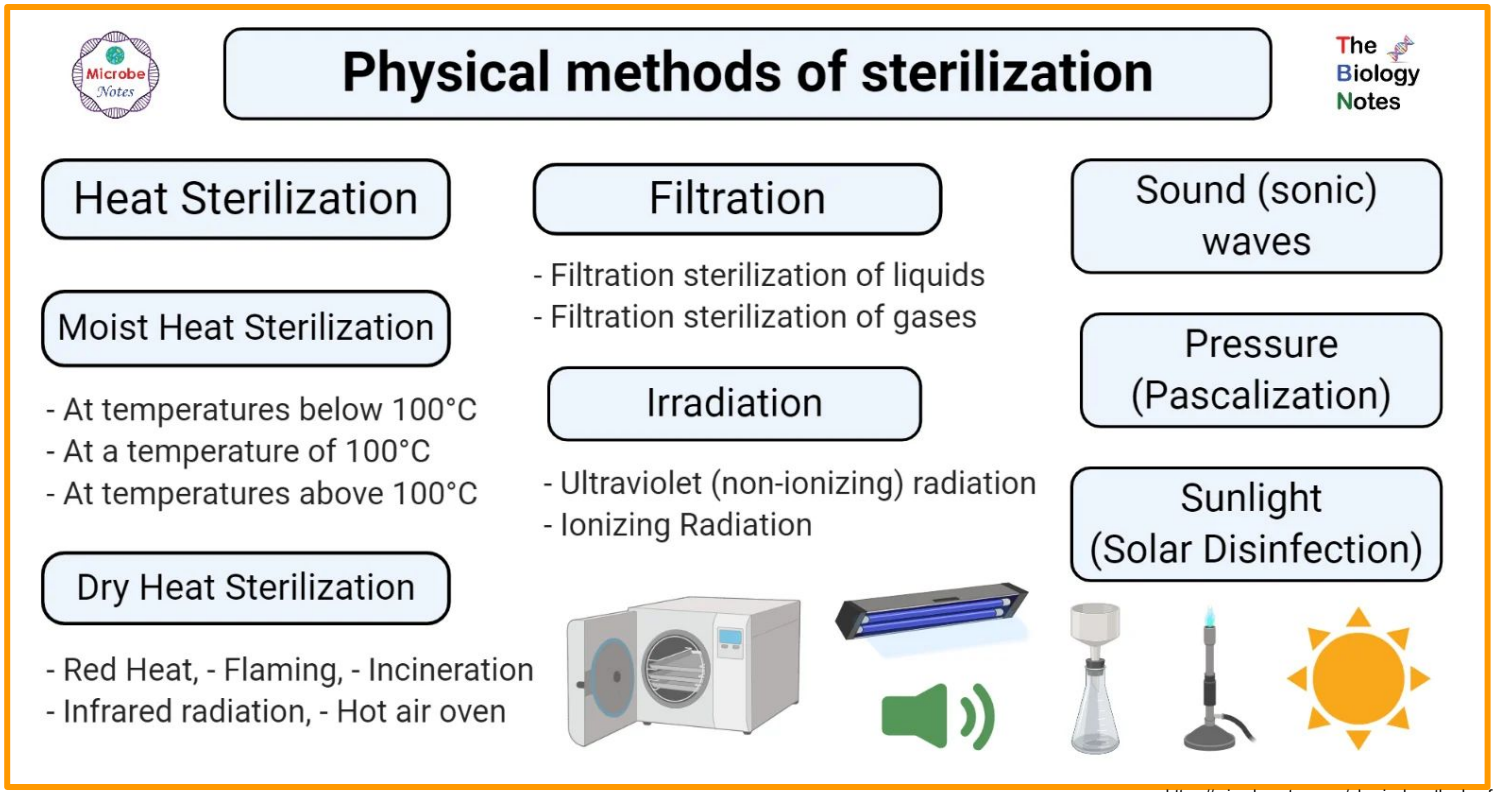
Liquid Sterilization

- Hydrogen peroxide
- Glutaraldehyde
- Hypochlorite



https://microbenotes.com/chemical-methods-of-sterilization/

3. Radiation – Ultraviolet (UV) radiation is effective to sterilize surfaces, but can not penetrate fluids easily. X-rays for liquids (costly/safety) because it can penetrate more deeply.



https://microbenotes.com/physical-methods-of-sterilization/#irradiation

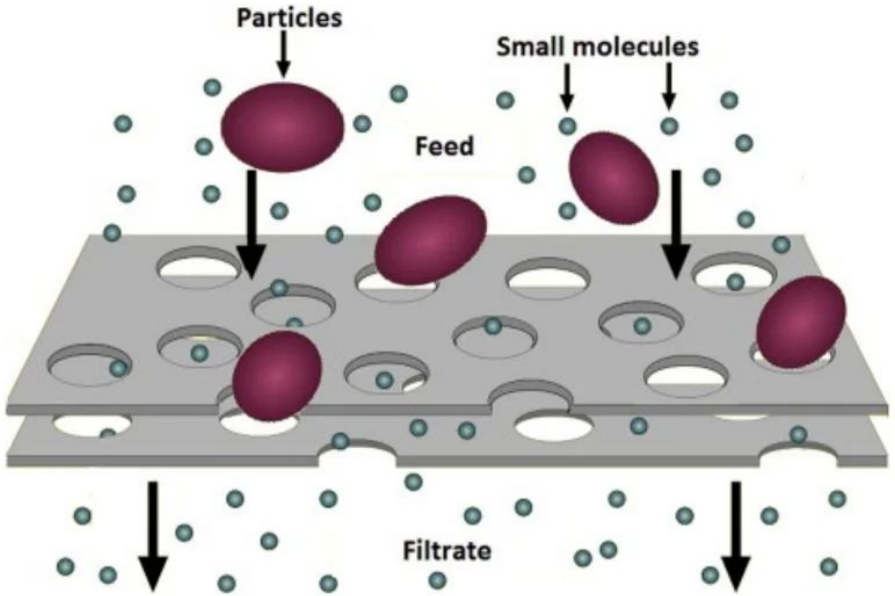
4. Filtration

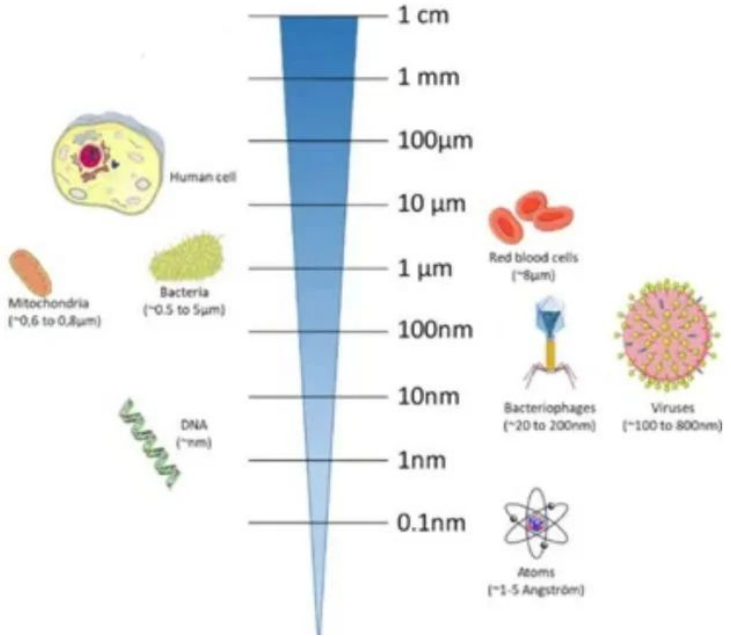
→ membrane filters having uniform micropores (<0.2m).

Not reliable as any defect in the membrane can lead to failure; virus can pass the filter.

→ depth filters of glass wool: rely on a combination of mechanisms (direct interception, electrostatic effects, diffusion and inertial effects) for the capture of particles.

Filtration Sterilization





https://microbiologynote.com/filtration-sterilization/

Sterilization of Objects, Products, and Packaging Surfaces and Their Characterization in Different Fields of Industry

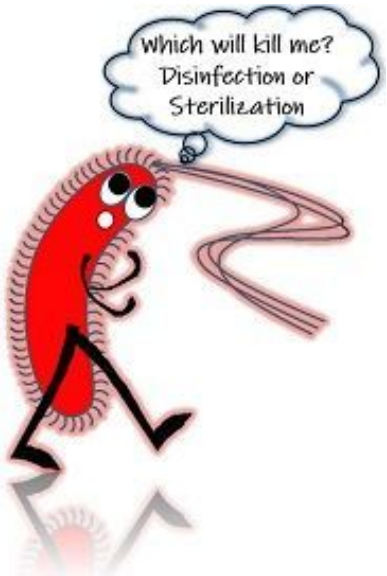
Cells or microbial life include viruses, bacteria, bacterial spores, fungi, protozoa, multicellular parasites, and contaminating eukaryotic cells. Biological entities include aberrant proteins (prions), endotoxins, or active deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

Disinfection is used to inactivate viable microorganisms to a level previously specified as being appropriate for a defined purpose. The validated processes that are used to render the product free from viable microorganisms are termed sterilization.

Disinfection vs Sterilization

Disinfection is a process that reduces microbial load, whereas **sterilization** completely removes or deactivates all present microorganisms, and upon process success the product is called sterile.

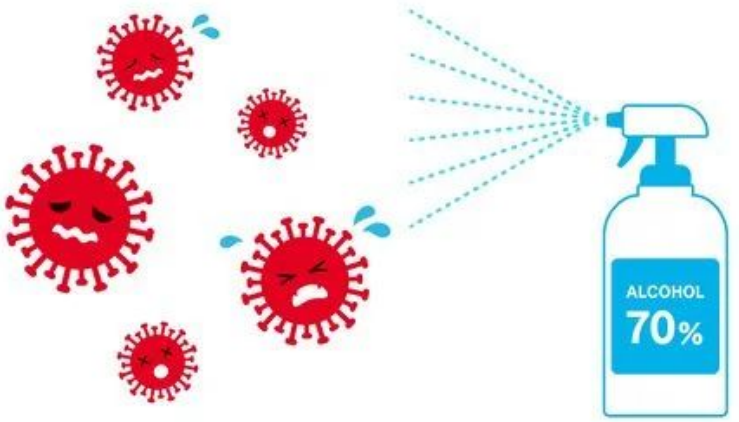
Both disinfection and sterilization methods are a part of the biodecontamination process.



https://biodifferences.com/difference-between-sterilization-and-disinfection.html

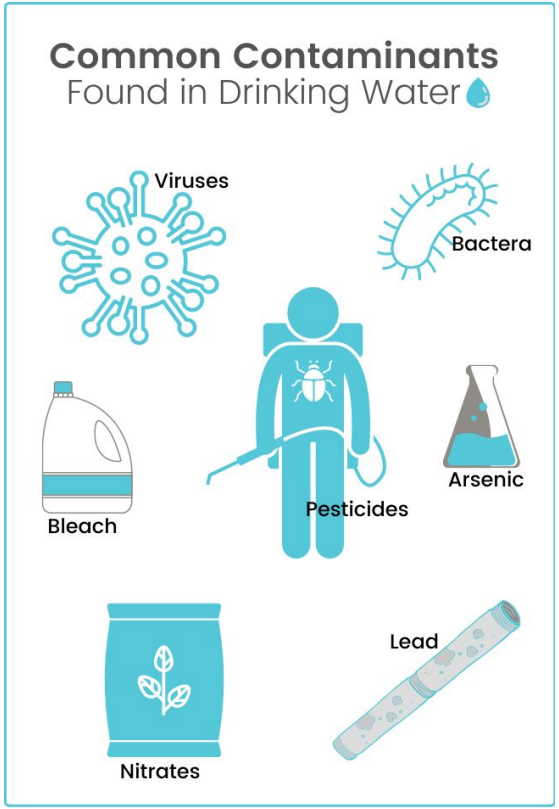
With relation to consumer products, the goal of sterilization differs from one industrial sector to another, as follows:

1) **medical and healthcare**
2) **pharmaceutics**
3) **food and beverages**
4) **cosmetics**



https://www.123rf.com/clipart-vector/germs.html

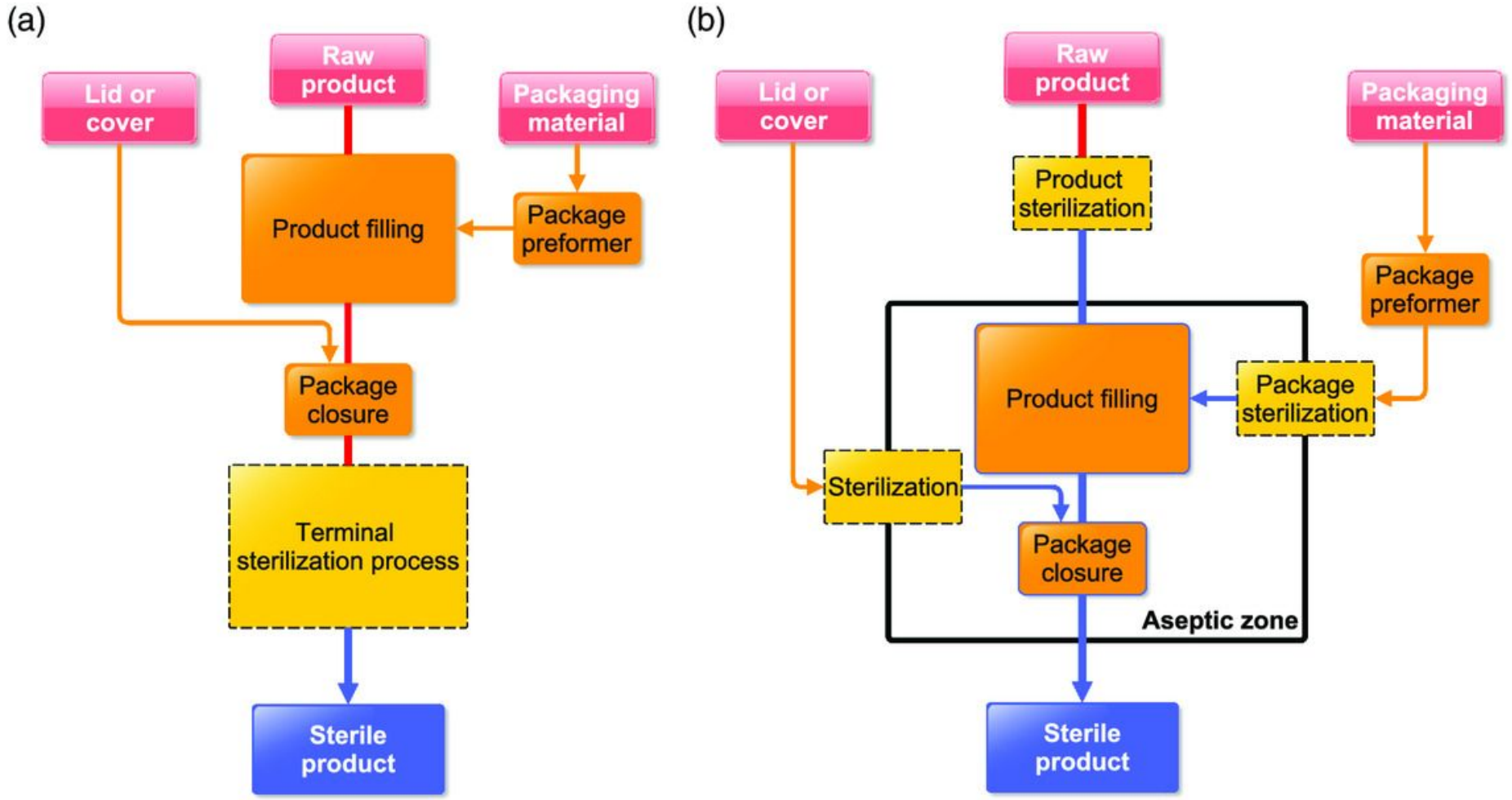
Contaminants can be “intrinsic,” meaning that they are already present in the untreated (raw) product or in/on packaging materials prior to processing, or “extrinsic,” such as those introduced by contamination during handling or manufacturing process.

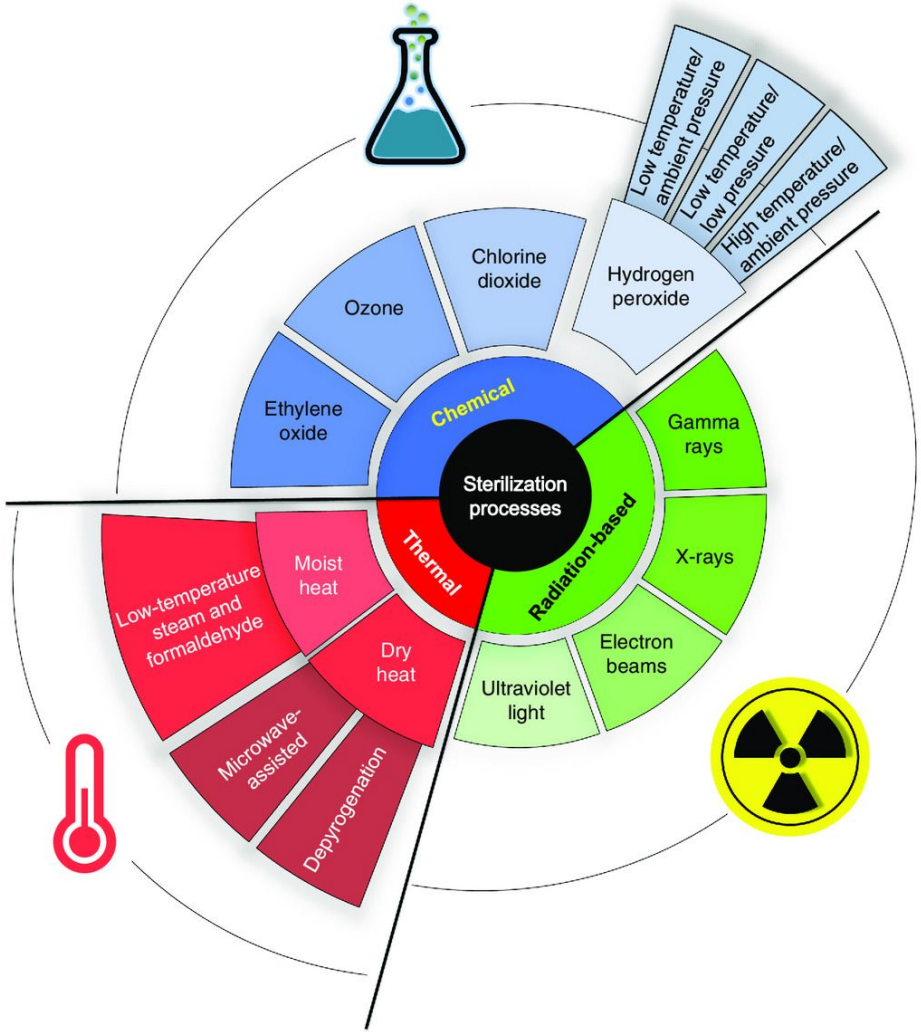


https://www.espwaterproducts.com/whats-in-your-drinking-water/

The differences between two packaging principles

Packaging of biologically sensitive products such as pharmaceuticals or food and beverages, or the handling of nonsingle - use surgical equipment, the target object is either terminally sterilized in its final package or aseptically filled


(a)
Lid or cover
Raw product
Packaging material
Product filling
Package preformer
Package closure
Terminal sterilization process
Sterile product
(b)
Lid or cover
Raw product
Packaging material
Product sterilization
Package preformer
Product filling
Package sterilization
Sterilization
Package closure
Aseptic zone
Sterile product


Sterilization processes
Chemical
Ozone
Chlorine dioxide
Hydrogen peroxide
Ethylene oxide
Low temperature/ ambient pressure
Low temperature/ low pressure
High temperature/ ambient pressure
Radiation-based
Gamma rays
X-rays
Electron beams
Ultraviolet light
Thermal
Moist heat
Dry heat
Low-temperature steam and formaldehyde
Microwave-assisted
Depyrogenation

Factors Affecting the Sterilization Efficacy

There are multiple factors that impact the design of the process and its efficacy, which are the type of biocontaminants, the location of bioburden, the surrounding conditions, and the exposure time to the sterilant.

For instance, in gaseous sterilization processes, a high level of humidity and a low sterilant gas temperature can decrease the sterilization efficacy.

Radiation Sterilization

Radiation sterilization facilities are still used in hospitals for medical purposes and as a pathogen control strategy in the food industry.

Compared with other sterilization methods such as heat, sterilization by radiation is characterized by short process time, its penetration capability through a wide range of substances, and simplicity of routine operation.

Disadvantages of this type of sterilization include its initial setup and maintenance costs, incompatibility with some packaging materials, and safety concerns related to the disposal of radioactive waste.

Gamma Rays

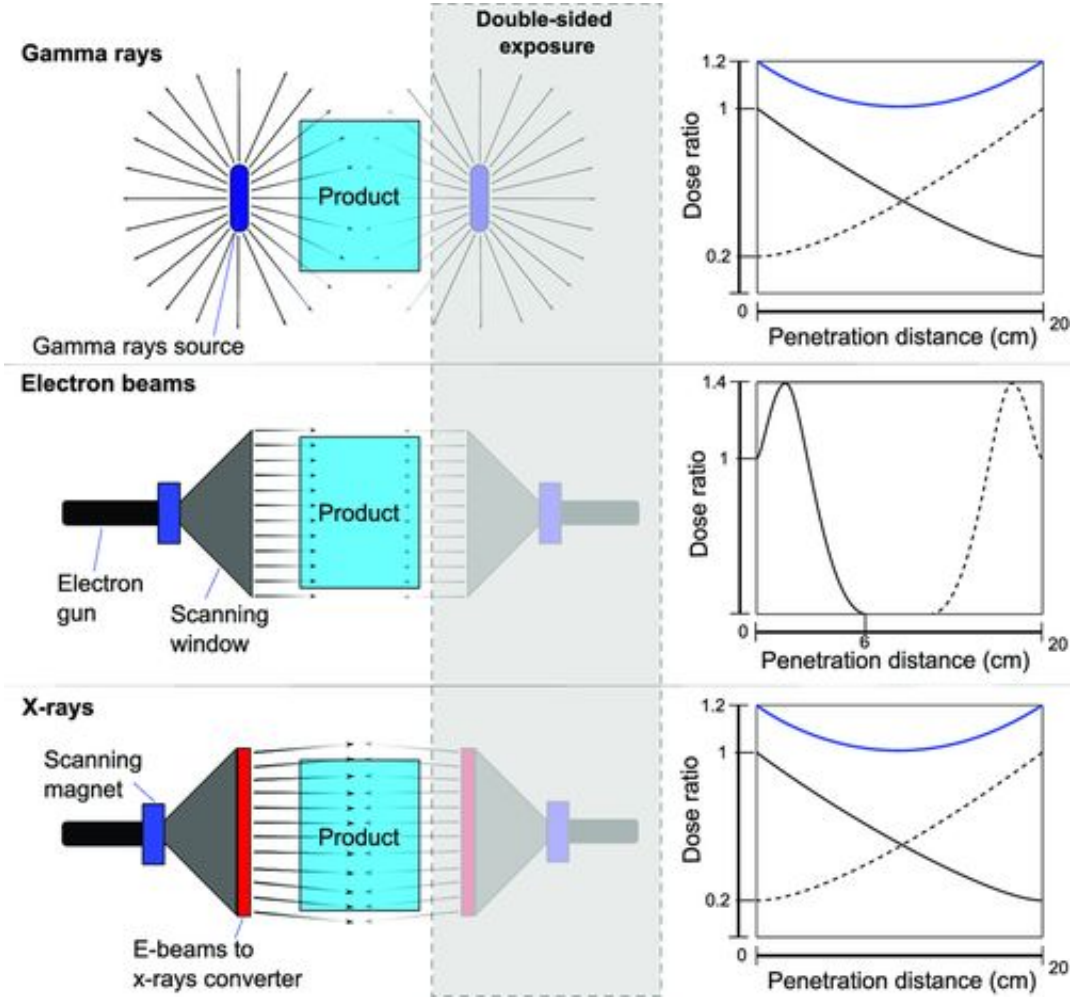
Gamma radiation, a form of ionizing radiation, became an established method for sterilizing single-use pharmaceutical, food, and cosmetic products. It is characterized by its ease of use and compatibility with diverse packaging materials.

The sterilization process occurs by exposing a product or package to a radioactive substance that releases high-energy photons.

The interaction of the photons with the molecules of microorganisms leads to the destruction of cellular nucleic acid, breakage in DNA strands, and the inhibition of protein synthesis.

One of the disadvantages of this type of sterilization is the requirement of a large technical facility that comes with a high capital investment.

Gamma radiation includes the release of volatile chemicals that affect the organoleptic properties and shelf-life of the product.



Thermal Sterilization

Heat causes an increase in the kinetic energy of atoms and molecules, which disrupts their bonds and their function within a biological entity such as a bacterium.

Dry heat form of sterilization is achieved by increasing the temperature of an object (a package, a bulk product, or a packaged product) through heat transfer from a heat source.

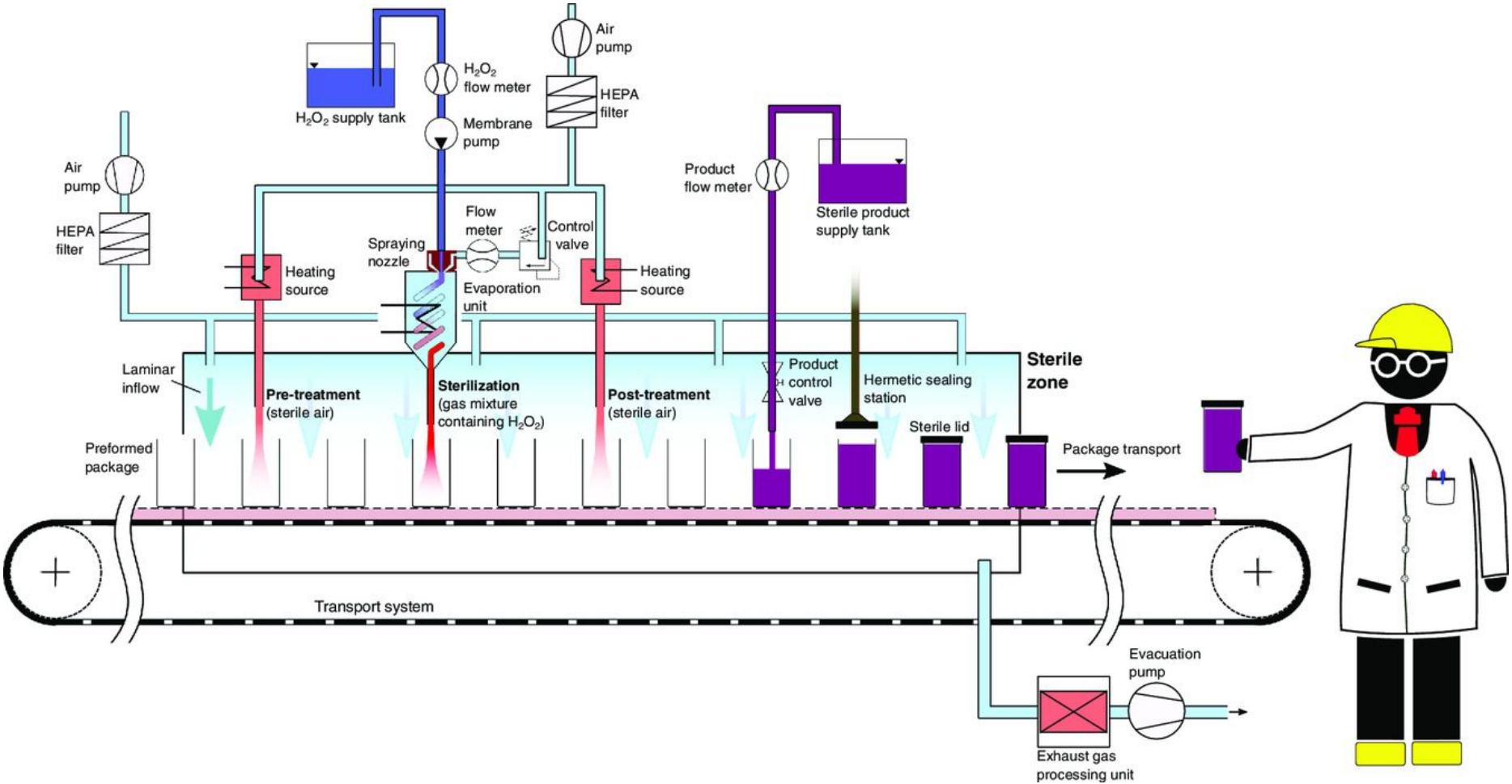
Materials such as nonaqueous products, metals, glass, powders, oils, and oil-based injectable pharmaceuticals and some polymers that do not lose their integrity under high temperatures can be sterilized with this method.

Chemical Sterilization

A chemical sterilant refers to a substance which is characterized by microbicidal properties. These properties include the ability of the chemical agent, or one of its derivatives, to interfere with genetic material (DNA or RNA) that deactivates one or multiple cellular functions, causing the death of the microorganism.

Other microbicidal courses of action are the inhibition of protein synthesis or enzymatic activity and direct damage to the cell membrane and cell wall.

The most widely used sterilants for the purpose of surface sterilization are ethylene oxide (C_2H_4O), ozone (O_3), chlorine dioxide gas (ClO_2), peracetic acid (CH_3CO_3H), and hydrogen peroxide (H_2O_2)


H_2O_2 supply tank
H_2O_2 flow meter
Membrane pump
Air pump
HEPA filter
Air pump
HEPA filter
Spraying nozzle
Flow meter
Control valve
Heating source
Heating source
Evaporation unit
Product flow meter
Sterile product supply tank
Laminar inflow
Pre-treatment (sterile air)
Sterilization (gas mixture containing H_2O_2)
Post-treatment (sterile air)
Product control valve
Hermetic sealing station
Sterile lid
Sterile zone
Preformed package
Package transport
Transport system
Evacuation pump
Exhaust gas processing unit

Biological Indicators

To validate the effectiveness of a sterilization process, standardized and regulated preparations of a selected group of resilient microorganisms are used.

These tests are termed “challenge tests” or “validation tests” and the used microorganisms are called “biological indicators.” What is being challenged is the ability of the sterilization process to deactivate these test microorganisms.

1) the strain shows high resistance to the specified sterilization process;
2) it is nonpathogenic;
3) it can be easily cultured and is easy to handle; and
4) the microorganism exhibits a long shelf-life and can be commercially distributed.

Sterilization method	Recommended test microorganisms [a]	Strain	Literature reference
Radiation (Gamma, e-beams, X-rays)	*Bacillus pumilus* [b]	ATCC 27142, CIP 77.25, NCTC 10327, NCIMB 10692	[109, 118]
	Bacillus cereus, *Lysinibacillus sphaericus* [c]	Not defined	[109]
UV light	*Cryptosporidium*, *Giardia lamblia*, viruses [d]	Not defined	[229]
Moist heat	*Geobacillus stearothermophilus* [e]	ATTC 7953, ATCC 12980, CIP 52.81, DSM 22, NCTC 10007, NCIMB 8157	[41, 109, 118, 208, 210, 230]
Low-temperature moist heat [f]	*Bacillus subtilus*	ATCC 35021	[230]
Low-temperature steam formaldehyde	*Geobacillus stearothermophilus*	ATTC 7953, ATCC 12980, ATCC 10149, DSM 6790, NCIB 8224	[123, 231]
Dry heat	*Bacillus atrophaeus* [g]	ATCC 9372, CIP 77.18, DSM 675, NCIMB 8058, NRRL B-4418	[109, 118, 210, 232]
	Bacillus subtilis	DSM 13019	[232]

Ethylene oxide	*Bacillus atrophaeus* [h)]	ATTC 9372, CIP 77.18, DSM 2277, NCTC 10073, NCIMB 8058, NRRL B-4418	[41, 109, 210, 233]
	Geobacillus stearothermophilus [i)]	ATTC 7953, CIP 52.81	[109]
Hydrogen peroxide [j)]	*Bacillus atrophaeus*	ATTC 9372, DSM 675	[208]
	Bacillus subtilis SA 22	NCA 72-52, DSM 4181	
	–		
	Geobacillus stearothermophilus	Not defined	[210]