Ecology of microorganisms. The effect on microbes of physical, chemical and biological factors
The concept of sterilization, disinfection, aseptic and antiseptic, conservation, their use in practice. Methods of sterilization. Equipment, mode, sterilizable material. Sterilization monitoring by physical, chemical and biological indicators

Microorganisms are affected by **physical, chemical and biological factors** of the environment.

Physical factors:

- Temperature
- Radiant energy
- Drying
- Ultrasonic
- Pressure
- Filtration

Chemical factors:

- pH
- Substances of different nature and concentration

Biological factors are the interrelationships of microorganisms with each other and with a macroorganism, the influence of enzymes, antibiotics.

Environmental factors can have a **positive effect** on microorganisms (growth stimulation), a **negative effect**, and also a **mutagenic effect**.

The negative effects are:

1) microbicidal - killing microorganisms;

2) microbostatic - inhibiting the growth of microorganisms.

Agents which kill cells are called **cidal** agents; agents which inhibit the growth of cells (without killing them) are referred to as **static** agents. Thus the term **bactericidal** refers to killing bacteria and **bacteriostatic** refers to inhibiting the growth of bacterial cells and so on. A **bactericide** kills bacteria, a **fungicide** kills fungi, and so on (**virulicide**).

These physical or chemical agents which either kill or prevent the growth of microorganisms are used for **"control of growth"** of microorganisms.

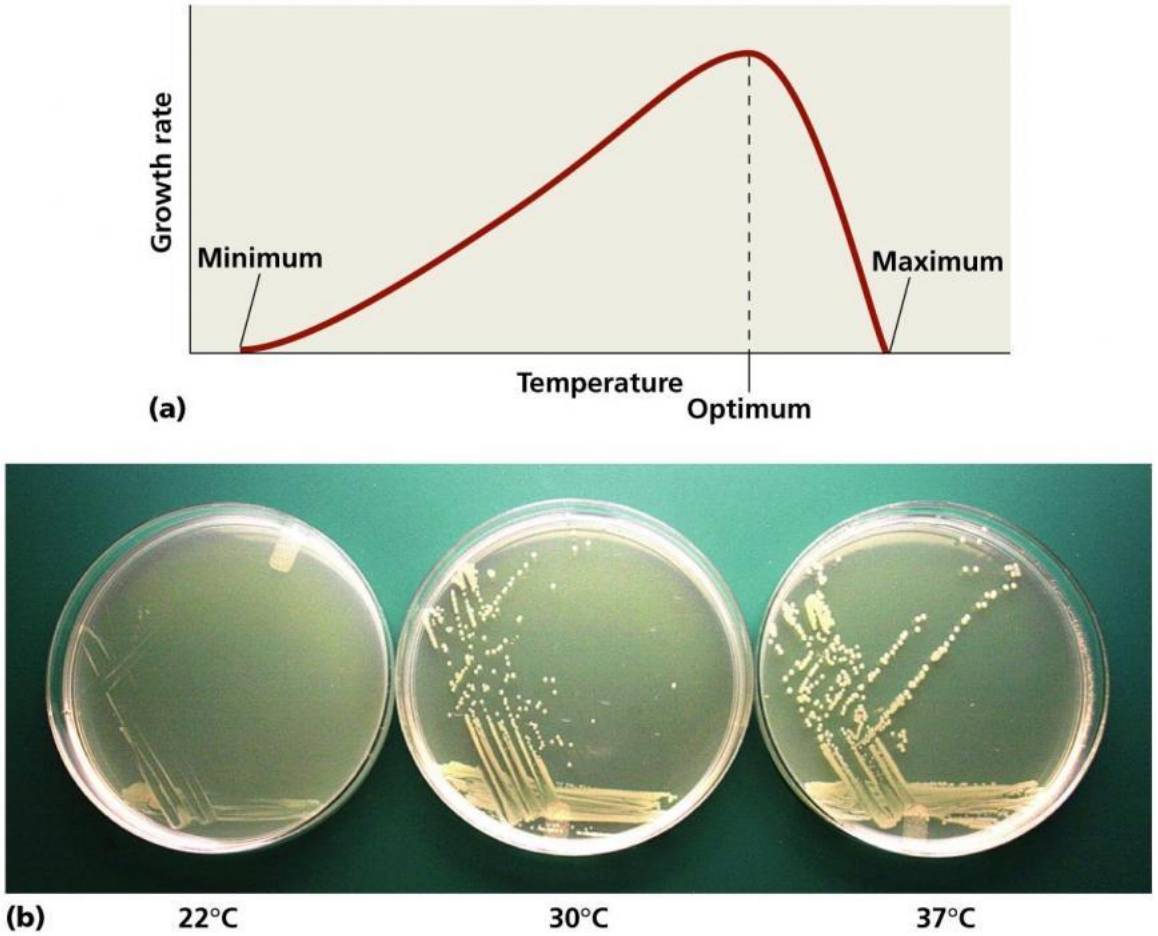
«Control of growth», as used here, means to prevent growth of microorganisms.

The control of microbial growth is necessary in many practical situations, and significant advances in agriculture, medicine, and food science have been made through study of this area of microbiology.

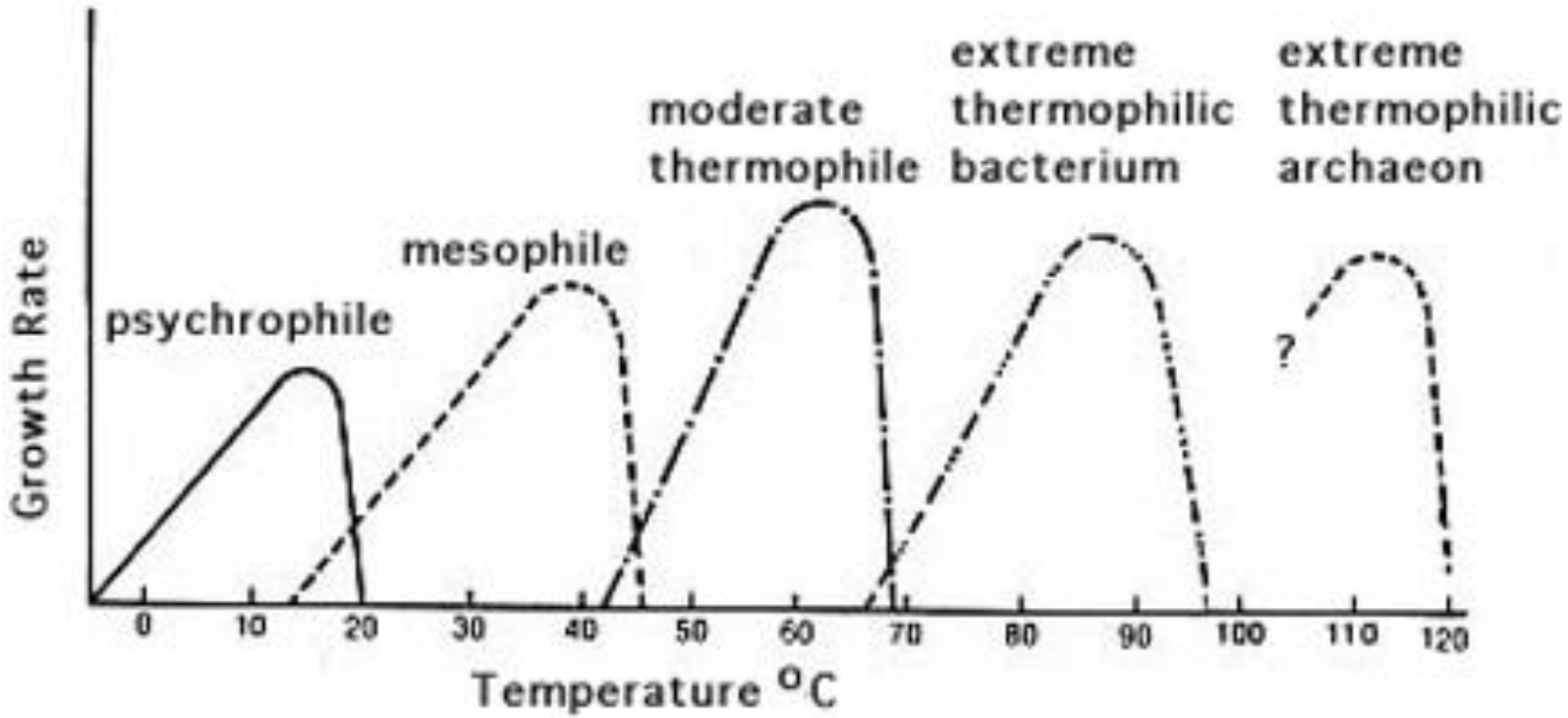
TEMPERATURE EFFECT ON MICROORGANISMS

Microorganisms have been found growing in virtually all environments where there is liquid water, regardless of its temperature. Subsequently, procaryotes have been detected growing around black smokers and hydrothermal vents in the deep sea at temperatures at least as high as +115°C. Microorganisms
have been found growing at very low temperatures as well. In supercooled solutions of H_2O as low as -20°C, certain organisms can extract water for growth, and many forms of life flourish in the icy waters of the Antarctic, as well as household refrigerators, near 0°C.

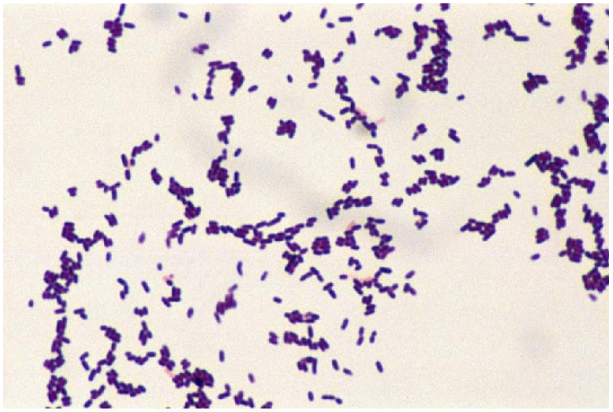
Considering the total span of temperature where liquid water exists, the procaryotes may be subdivided into several subclasses on the basis of one or another of their cardinal points for growth. For example, organisms with an optimum temperature (T) near +37°C (the body temperature of warm-blooded animals) are called **mesophiles.** Organisms with an optimum T between about +45°C and +70°C are **thermophiles.** Some *Archaea* with an optimum T of +80°C or higher and a maximum T as high as +115°C, are now referred to as **extreme thermophiles** or **hyperthermophiles.** The cold-loving organisms are **psychrophiles** defined by their ability to grow at 0°C, A variant of a psychrophile (which usually has an optimum T of +10-15°C) is a **psychrotroph,** which grows at 0°C but displays an optimum T in the mesophile range, nearer room temperature. Psychrotrophs are the scourge of food storage in refrigerators since they are invariably brought in from their mesophilic habitats and continue to grow in the refrigerated environment where they spoil the food. Of course, they grow slower at +2°C than at +25°C.



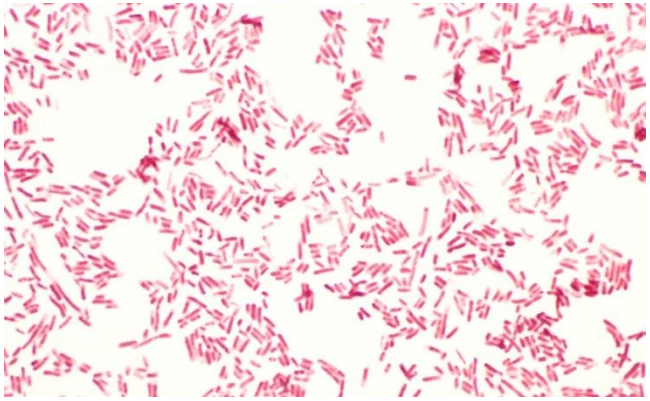
Investigation optimal temperature of the species



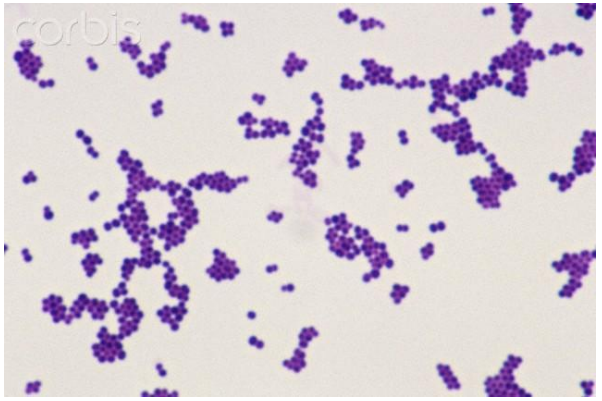
Subclasses on the basis of one or another of their cardinal points for growth



L. Monocytogenes (Gram Stain)



E. Coli (Gram Stain)



S. Aureus (Gram Stain)

Some notable mesophiles include *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*. Other examples of species of mesophiles are *Clostridium kluyveri*, *Pseudomonas maltophilia*, *Thiobacillus novellus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. Different types of diseases and infections typically have pathogens from mesophilic bacteria such as the ones listed above.

Archaea were first found in extreme environments, such as volcanic hot springs. Pictured here is Grand Prismatic Spring of Yellowstone National Park.

Favorable action of the optimum temperature is used in the cultivation of microorganisms for the purpose of laboratory diagnosis, preparation of vaccines and other preparations.

Low temperature (refrigeration and freezing)

most organisms grow very little or not at all at 0°C. Store perishable foods at low temperatures to slow rate of growth and consequent spoilage (e.g. milk). Low temperatures are not bactericidal. Psychrotrophs, rather than true psychrophiles, are the usual cause of food spoilage in refrigerated foods. For example, *Listeria monocytogenes* is of great concern in refrigerated foods.

The mechanism of action of low temperatures - inhibition of metabolic processes, growth and reproduction of microorganisms and transition to a state of suspended animation.

High temperature has a killing effect. The killing effect of high temperature (above the maximum) is used for sterilization. The mechanism of action is the denaturation of the protein (enzymes), damage to the ribosomes, the violation of the osmotic barrier. The **psychrophils** and **mesophiles** are the most sensitive to the action of high temperature. Specific resistance is shown by bacterial spores.

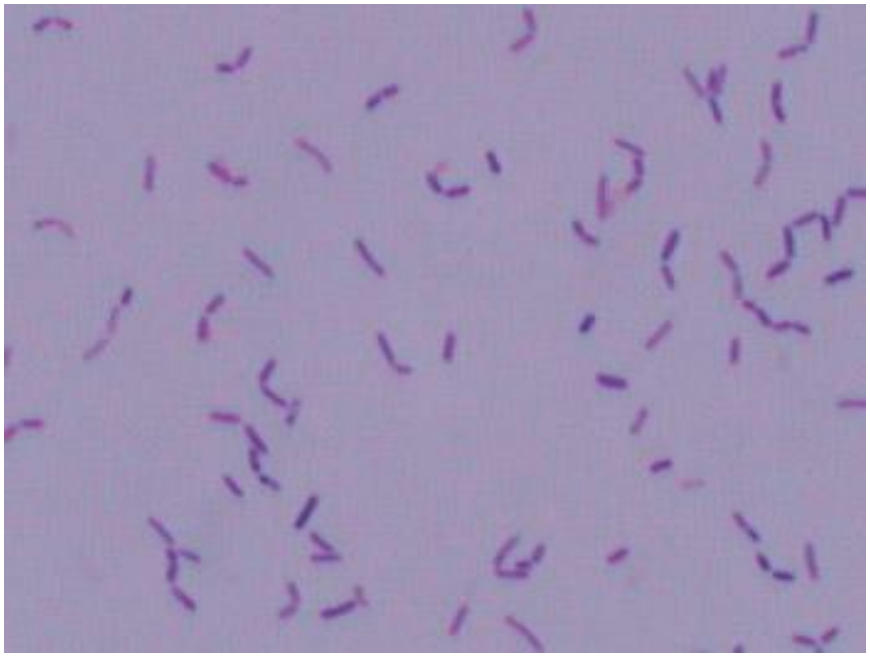
The lethal **temperature** varies in microorganisms. The **time** required to kill depends on the number of organisms, species, nature of the product being heated, pH, and temperature. Whenever heat is used to control microbial growth inevitably **both time and temperature are considered.**

RADIATION

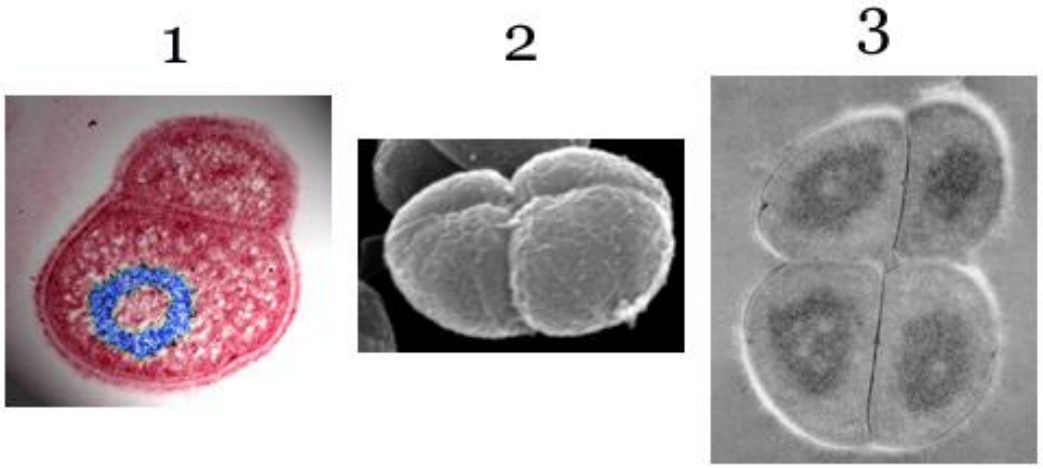
Electromagnetic radiation of various types bombards our world. As the wavelength of electromagnetic radiation decreases, the energy of the radiation increases – gamma rays and X rays are much more energetic than visible light or infrared waves. Sunlight is the major source of radiation on the earth. It includes visible light, ultraviolet radiation, infrared rays and radio waves. Most life is dependent on the ability of photosynthetic organisms to trap the light energy of the sun as visible light.

RADIATION

Many forms of electromagnetic radiation are very harmful to microorganisms. Ionizing radiation, radiation of very short wavelength or high energy can cause atoms to lose electrons or ionize. The two major forms of ionizing radiation, X rays which are artificially produced and gamma rays which are emitted during radioisotope decay. Low levels of ionizing radiation will produce mutations, higher levels are directly lethal. Some prokaryotes like *Deinococcus radiodurans* and bacterial endospores are resistant.

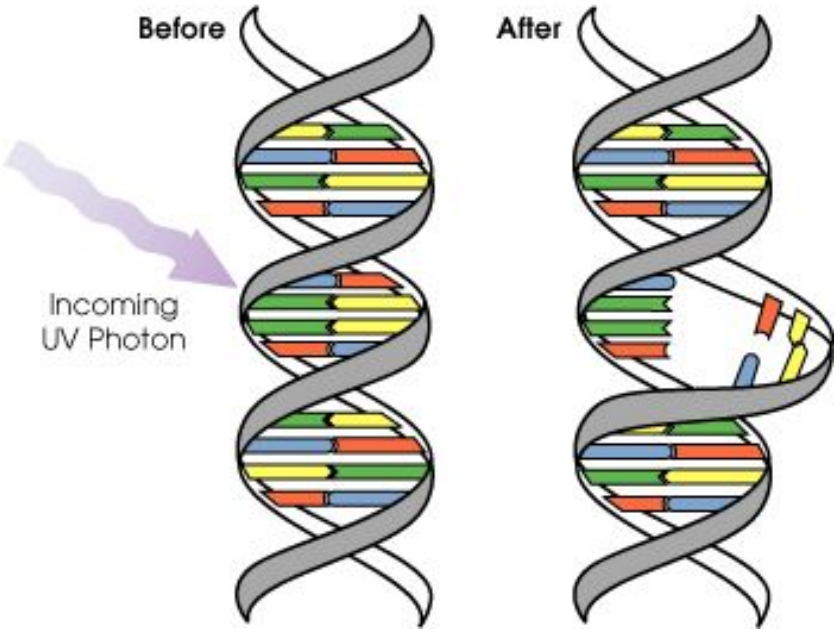


Gram Stain of isolated UV resistant organism *Deinococcus radiodurans* from air sample grown on NA + 7.5% NaCl plate incubated at 30° C for 5 days. Shows small gram positive rods (1000X)

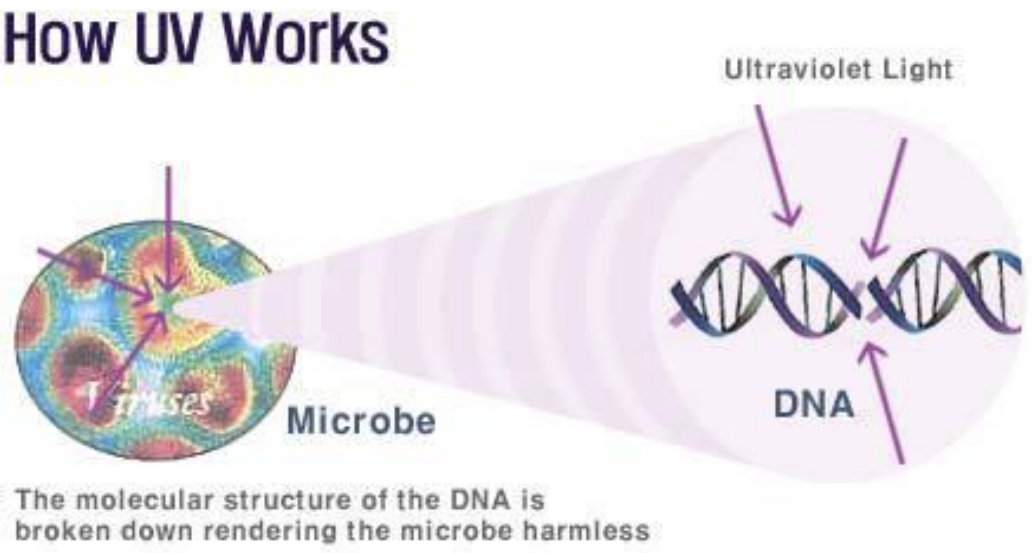


The electron micrographs above show some of the unusal features of *D. radiodurans.* The four compartments of a single cocci can be seen in 2 and 3. In 1 and 3, the tightly coiled DNA torus is visible. Source: Weizmann Institute of Science wis-wander.weizmann.ac.il

Effects of irradiation on microorganisms: usually destroys or distorts nucleic acids; it breaks hydrogen bonds, oxidises double bonds, destroys ring structures and polymerizes some molecules.



The mechanism of the damaging effect of UV rays: the formation of dimers of thymine in the DNA molecule, which stops cell division and is the main cause of their death. The damaging effect of UV rays is more pronounced for microorganisms than for animals and plants.



The mechanism of ionizing radiation (X-ray)

It has a powerful penetrating effect and damages the cellular genome. **The mechanism of the damaging action**: the ionization of macromolecules, which is accompanied by the development of mutations or cell death. At the same time, lethal doses for microorganisms are several orders of magnitude higher than for animals and plants.

Ultraviolet light is **usually used for sterilization** (commonly used to sterilize the surfaces of objects), although X-rays and microwaves are possibly useful. Many spoilage organisms are easily killed by irradiation. In **some** parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent.

Drying (removal of H_2O - Desiccation)

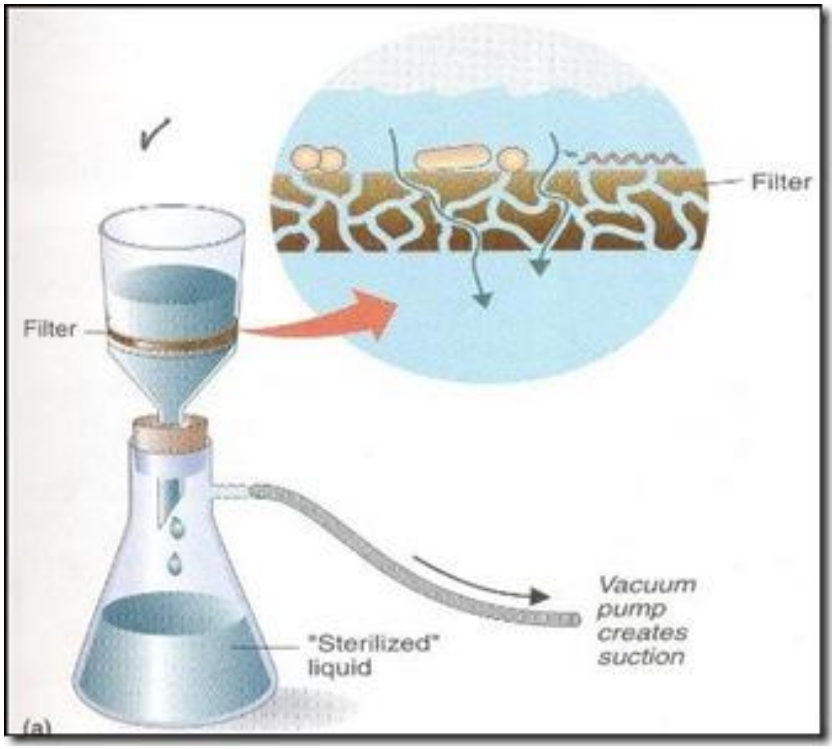
Most microorganisms cannot grow at reduced water activity (Aw < 0.90). Often used to preserve foods (e.g. fruits, grains, etc.). Methods involve removal of water from product by heat, evaporation, freeze-drying, and addition of salt or sugar.

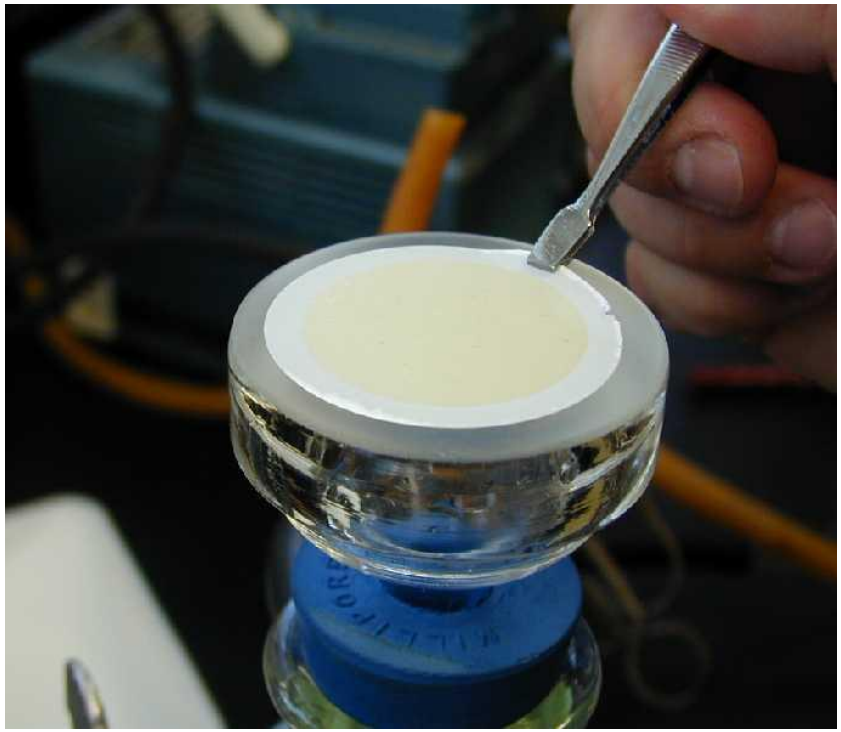
Drying from the frozen state under vacuum is **lyophilization or freeze-drying**. It is used to preserve cultures of microorganisms that in this state for years (10-20 years) do not lose their viability and do not change properties. Microorganisms are in this state in anabiosis. Lyophilization is used in the **production of bacterial preparations from living microorganisms: eubiotics, phages. live vaccines.**

Lyophilization Freeze Dryer System

FILTRATION

involves the physical removal (exclusion) of all cells in a liquid or gas, especially important **to sterilize** solutions which would be denatured by heat (e.g. antibiotics, injectable drugs, amino acids, vitamins, etc.)



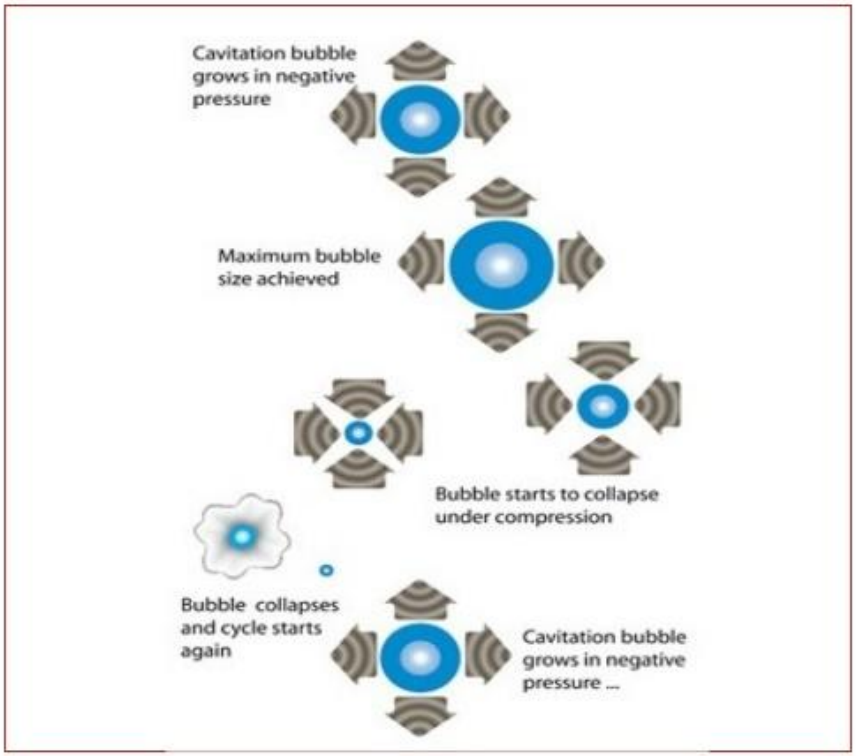


ULTRASONIC

Ultrasonic (sound waves of 20 thousand Hz) has a **bactericidal effect**. Ultrasonic cleaning of dental instruments is widely used.

Ultrasonic Cleaning Process

Ultrasonic cleaner uses high frequency wave that agitates liquid within the sink (tank) creating a rapid formation and collapse of bubbles; also known as cavitation.

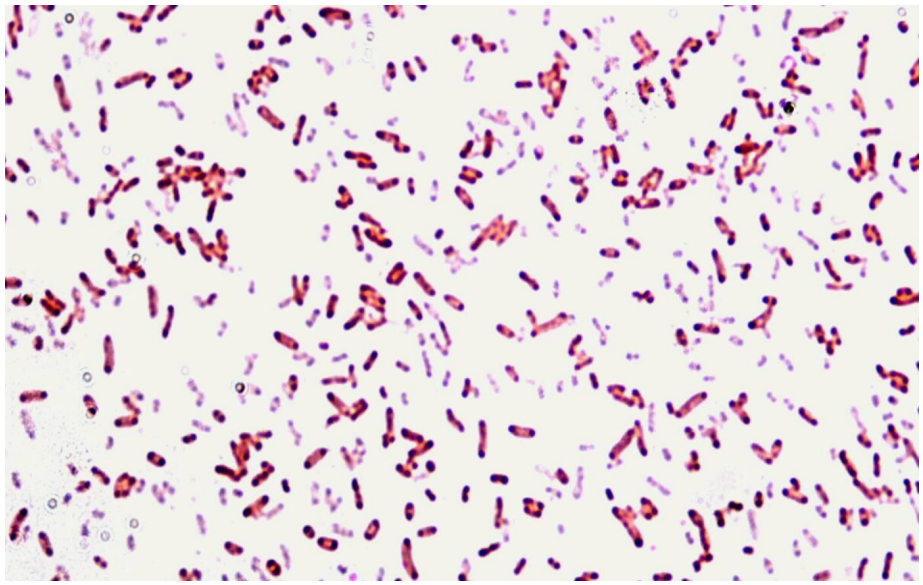


As the bubbles collapse during the cavitation process the cleaning solution rushes into hard to reach areas of objects submerged in the sink (tank). This process is highly effective to gently removing all contaminants and dirt from both the surface and hard to reach areas of intricately shaped objects.

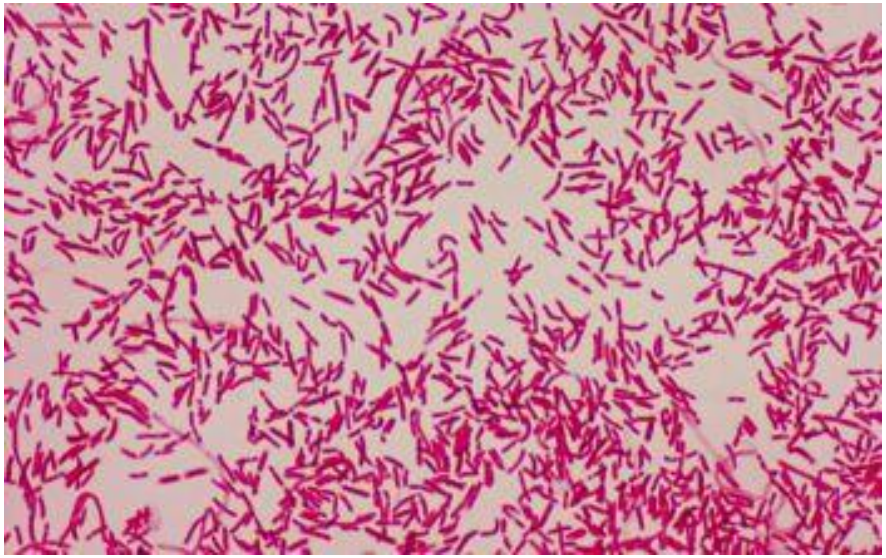
Mechanism: **the formation of cavitation cavities in the cytoplasm** of the cell, which are filled with liquid vapors and in them pressures of up to 10,000 atm occur, which leads to the formation of highly reactive hydroxyl radicals, to the disintegration of cellular structures and the depolymerization of organelles, and the denaturation of molecules.

PRESSURE

Most organisms on land or on the surface of water is always subjected to a pressure of 1 atm. The hydrostatic pressure can reach 600 to 1100 atm in the deep sea. Despite these extremes, bacteria survive and adapt. Many are barotolerant. Some bacteria in the gut of deep sea invertebrates such as amphipods and holothurians are truly barophilic and grow more rapidly at high pressures (Ex. *Photobacterium, Shewanella, Colwellia*).



Photobacterium damselae ssp. Piscicida is a gram-negative rod-shaped bacterium that causes disease in fish
1250x magnification



Shewanella putrefaciens lives in the environment and in food products, does not belong to the normal flora of the human being

ACTION OF CHEMICAL FACTORS ON MICROORGANISMS.

Depending on the nature, concentration and duration of the action, chemicals stimulate growth (they are used as energy sources), have a microbicidal, microbostatic, mutagenic effect or may be indifferent to vital processes.

For example, a 0.5-2% glucose solution is a food source for microbes, and a 20-40% solution has a depressant effect.

For microorganisms, the **optimal pH** (potential of hydrogen) of the medium is required. **pH** refers to the acidity or alkalinity of a solution. It is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration.

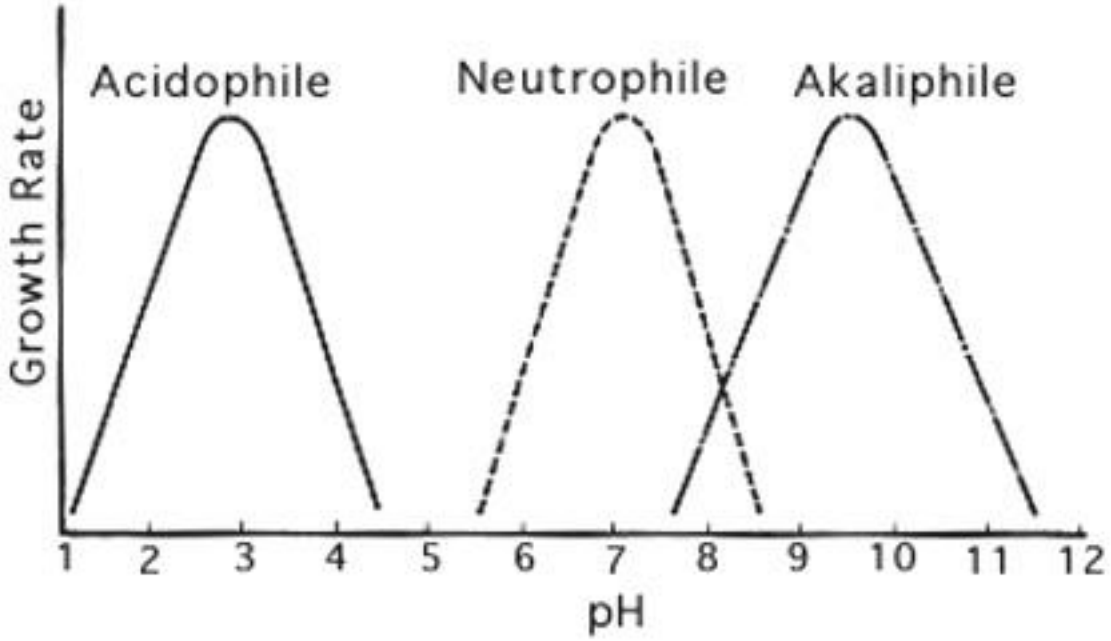
$pH = -\log [H^+] = \log (1/H^+)$

More precisely it is the negative of the base 10 logarithm of the activity of the hydrogen ion.

The pH scale ranges from 1.0 to 14.0 and most microorganisms grow vary widely from pH 0 to 2.0 at the acid end to alkaline lakes and soil that may have pH values between 9.0 and 10. The pH can affect the growth of microorganisms and each species has a definite pH growth range and pH growth optimum.

Classification of Bacteria According to pH

- ✔ **Neutrophiles** - grow between pH 5.5 to 8.0.
- ✔ **Alkaliphiles** - grow between pH range of 7.5 to 14.
- ✔ **Acidophiles** - grow between pH 0 and 5.5. Bacteria prefer media of pH near neutrality, and usually cannot tolerate pH values much below 4-5.



For most symbionts and causative agents of human diseases - a neutral, slightly alkaline or slightly acidic environment. With growth, the pH shifts more often to the acidic side, the growth of microorganisms is suspended at the same time. And then death comes. **Mechanism**: denaturation of enzymes by hydroxyl ions, disrupting the plasma membrane and membrane transport proteins.

Antimicrobial chemicals are used for **disinfection, sterilization, antisepsis and conservation.**

The use of physical and chemical methods in Microbial control.

Although microorganisms are beneficial and necessary in human well-being, microbial activities have undesirable consequences such as food spoilage and disease. To minimize their destructive effects, it is essential to kill a wide variety of microorganisms or inhibit their growth. The goal is twofold, to destroy pathogens and prevent their transmission and to reduce or eliminate microorganisms responsible for the contamination of water, food and other substances.

Sometimes it is necessary to eliminate the microorganisms completely from an object, whereas sometimes only partial destruction may be required in other situations.

Types of Microbiological Control

Sterilization is the complete destruction or elimination of all viable organisms (in or on an object being sterilized). There are no degrees of sterilization: an object is either sterile or not. Sterilization procedures involve the use of heat, radiation or chemicals, or physical removal of cells. When sterilization is achieved by a chemical agent, the chemical is called a **sterilant.**

Disinfection – is the killing, inhibition or removal of microorganisms that may cause disease In the external environment (on (in) the objects of the environment). Disinfection also involves the use chemicals or physical removal of cells.

Disinfectants are agents, usually chemical used to carry out disinfection and does not necessarily sterilise an object because viable spores and few microorganisms may remain. **Sanitization** is closed related to disinfection.

Sanitization

The process of reducing microbial contamination to an acceptable “safe” level. The process of cleaning objects without necessarily going through sterilization.

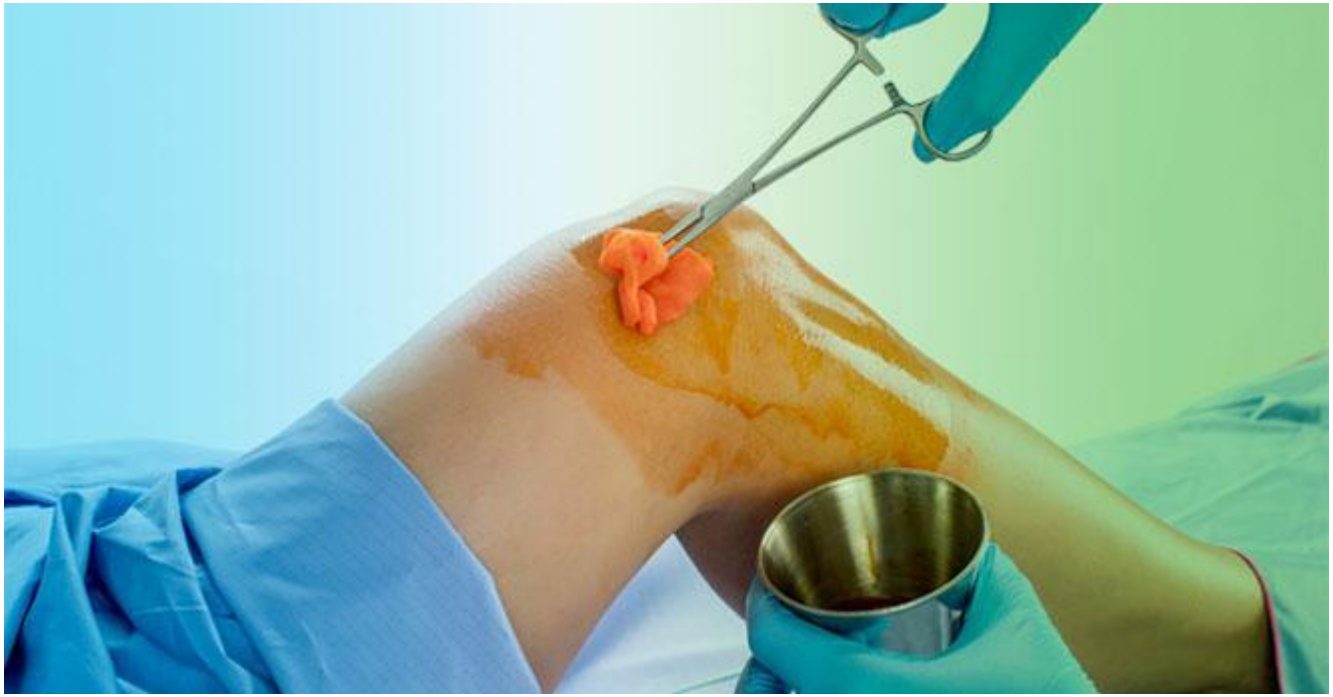
Decontamination

The killing of organisms or removal of contamination after use, with no quantitative implication, generally referring to procedures for making items safe before disposal.

Preservation

It is inhibition of growth of microorganisms in/on objects. It is sometimes necessary to control microorganisms on living tissue with chemical agents.

Antisepsis is the killing or inhibition growth of microorganisms in the living tissues, i.e. antisepsis is the prevention of infection or sepsis. Antisepsis can involve mechanical, chemical or physical modes of removal of microbial cells.



Chemical method is accomplished with **antiseptics.** These chemical agents are applied to living tissue and they prevent infection by killing or inhibiting pathogen growth or they reduce the total microbial population.

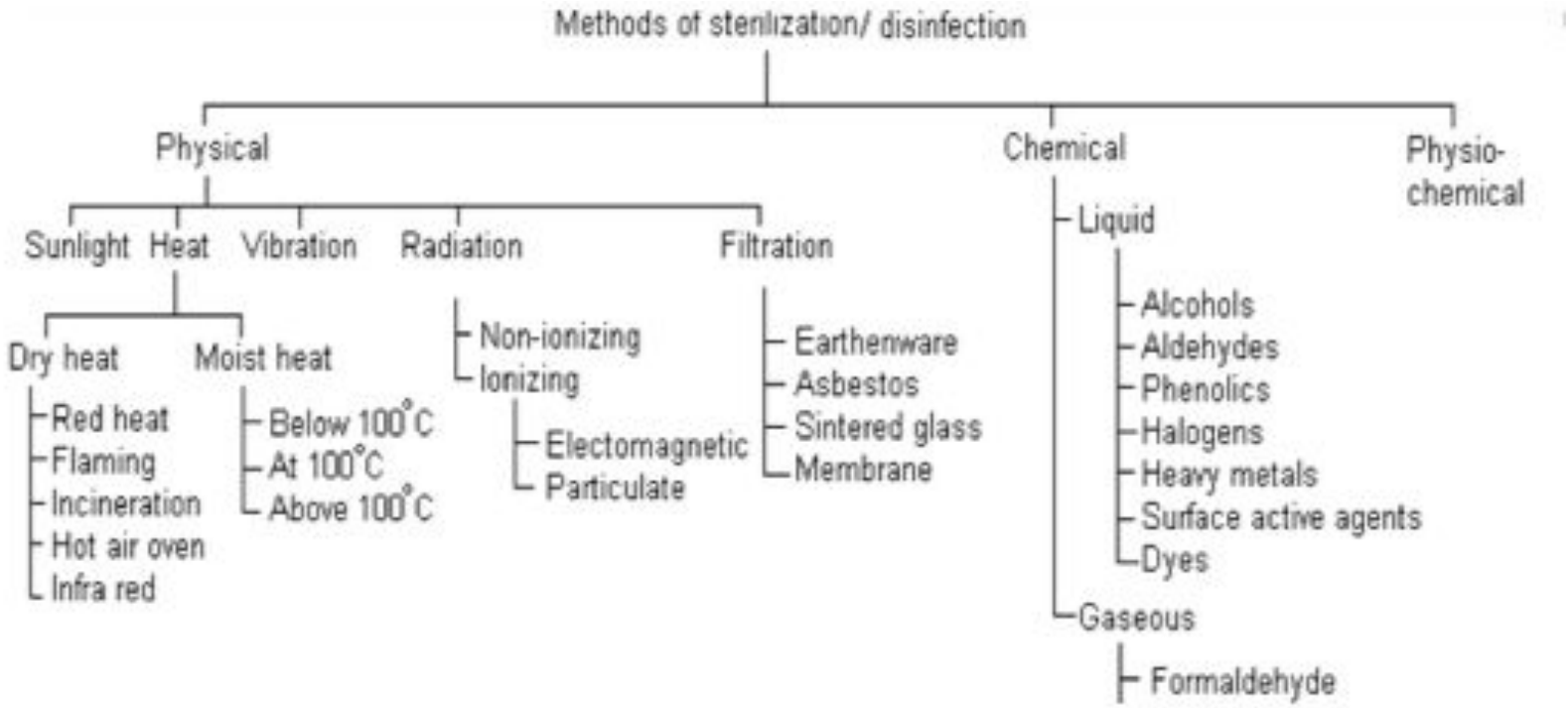
Substances that kill organisms often have the suffix – **cide**. **Germicide:** An agent that destroys microorganisms, particularly pathogenic microorganisms.

A disinfectant or antiseptic can be effective against a specific group and may be called a **bactericide, fungicide, algicide and viricide.** Other chemicals do not kill, but they do prevent growth, and if these are removed, growth will resume. Their names end in –static like, **bacteriostatic** and **fungistatic.**

Unlike antibiotics, **antiseptics and disinfectants** have a **nonspecific action** against a wide range of microorganisms, whereas **antibiotics have specificity and selectivity** for microorganisms. Antibiotics and chemotherapeutic drugs act in concentrations of 100-100 times less than antiseptics and disinfectants.

STERILIZATION METHODS

There are 3 groups of methods of sterilization: **physical, chemical and physico-chemical methods.**



Physical methods include

- High temperature (heat)
- UV irradiation, ionizing irradiation
- Ultrasound
- Filtration through sterile filters

HEAT

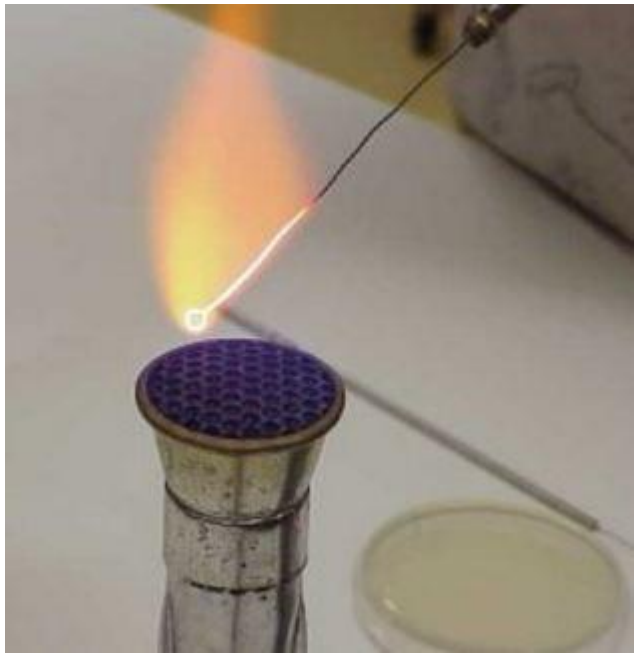
Heating is still one of the most popular ways to destroy microorganisms. Fire and boiling water have been used since the time of Greeks for sterilization and disinfection. For sterilization always consider type of heat, time of application and temperature to ensure destruction of all microorganisms. Endospores of bacteria are considered the most thermoduric of all cells so their destruction guarantees sterility. Either **moist heat** or **dry heat** may be applied.

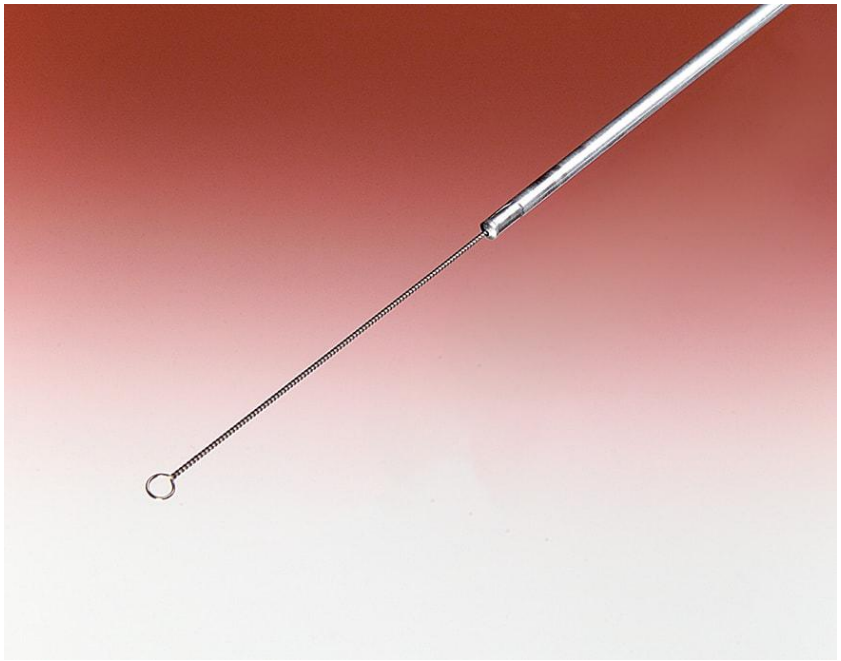
TYPES OF HEAT STERILIZATION

1. Flaming is the process of exposing metallic device like the needle, scalpels, scissors to flame for few minutes. The fire burns the microbes and other dust on the instrument directly.

TYPES OF HEAT STERILIZATION

2. **Incineration** is done especially for bacteriological loops used in microbe cultivation. The metallic end of the loop is heated to red hot on the flame. This exposure kills all the germs.





TYPES OF HEAT STERILIZATION

3. **Boiling:** + 100°C for 30 minutes. Kills everything except some endospores (actually, for the purposes of purifying drinking water +100°C for five minutes is probably adequate though there have been some reports that *Giardia* cysts can survive this process). Exposure to boiling water for 10 min is sufficient to kill or destroy vegetative cells and eukaryotic spores., but not enough to kill or destroy bacterial endospores. To kill endospores, and therefore **sterilize** the solution, very long or **intermittent boiling** is required.

TYPES OF HEAT STERILIZATION

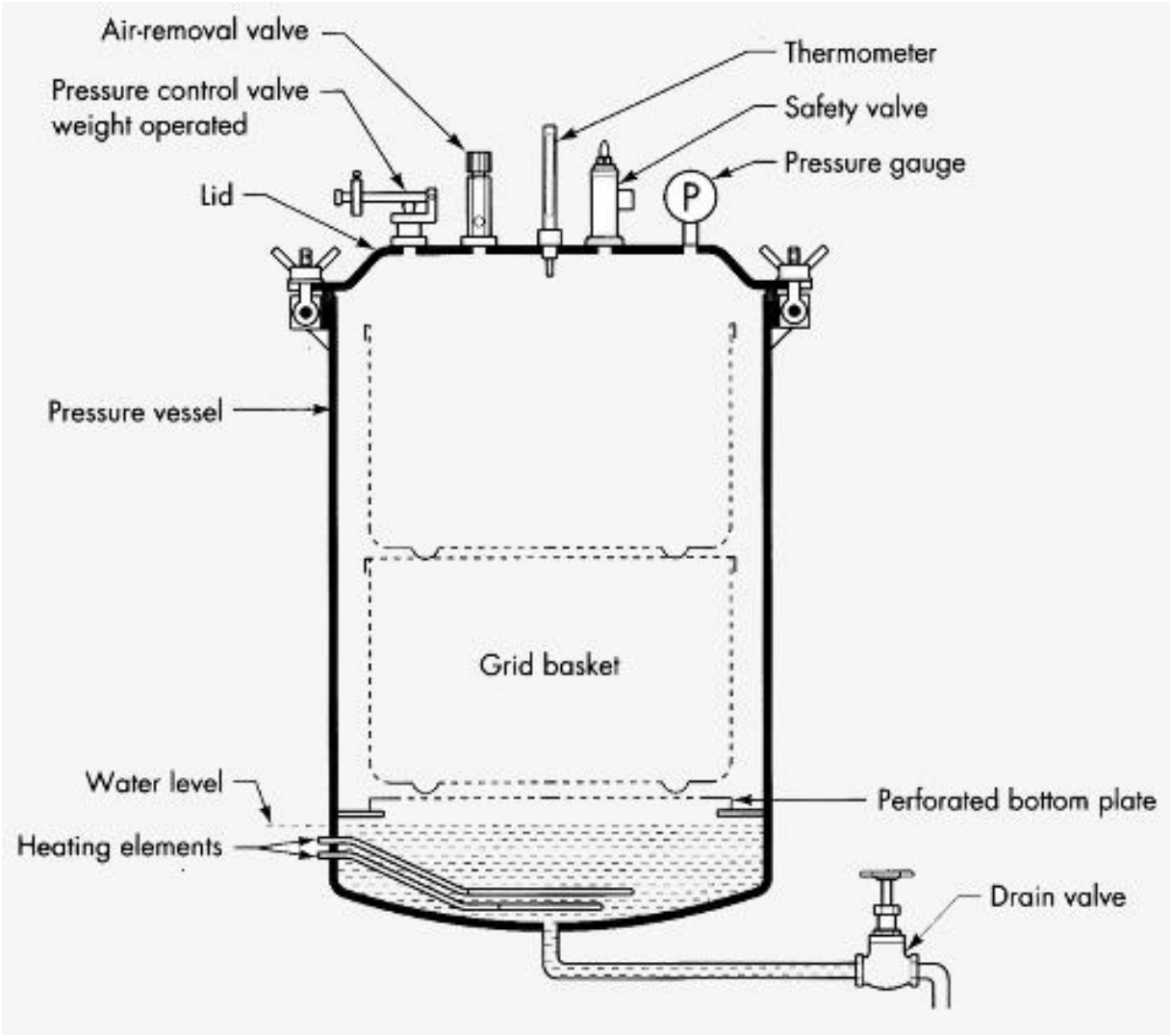
In order to destroy bacterial endospores, moist heat sterilization must be carried out at temperatures above 100°C and this requires the use of saturated steam under pressure. This can be carried out with an **autoclave** (Chamberland, 1884).

Autoclaving (steam under pressure or pressure cooker): +121°C for 15-20 minutes (15 pounds pressure). Water is boiled to produce steam, which is released through the jacket and into the autoclave's chamber. Hot, saturated steam enters the chamber and the desired temperature and pressure, usually 121°C and 15 pounds is reached. At this temperature saturated steam destroys all vegetative cells and endospores. **Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins. It also may disrupt cell membranes.** Good for sterilizing almost anything, but heat-labile substances will be denatured or destroyed.

AUTOCLAVE

The autoclave consists of 2 metal cylinders, inserted into each other with a hermetically sealed cover, having screws. It is equipped with a pressure gauge, steam valve, safety valve, water-glass. External cylinder - water-vapor chamber, internal - sterilization chamber. In the upper part of the sterilization chamber there is an opening through which steam flows from the water-steam chamber into sterilization chamber. The pressure gauge serves to determine the pressure in the sterilization chamber. There is a definite relationship between pressure and temperature: 121°C - 15 pounds (0.5 atm - 112 ° C, 1-01.1 atm - 119-121 ° C, 2 atm - 134 ° C). Safety valve - to protect against excessive pressure: when the pressure rises above the preset, it opens and releases excess steam.

AUTOCLAVE DIAGRAM



Operating procedure. The water is poured into the autoclave, the level of which is controlled by the water glass. The material is placed in the sterilization chamber and the cover hermetically is closed. The steam valve is open. Turn on the heat. After the boiling of water, the steam valve is closed only when all air is expelled (the steam flows continuously with a strong, dry spray). If the valve is closed earlier, the pressure gauge reading will not match the desired temperature. After the valve is closed, the pressure in the boiler gradually increases. The beginning of sterilization is the moment when the needle of the pressure gauge shows the preset pressure.

After the sterilization, the heating is stopped and the autoclave is cooled before returning the pressure gauge needle to 0. If steam is released earlier, the liquid may boil up due to a rapid change in pressure and push out the plugs (***objects will lose their sterility***). When the cursor of the pressure gauge returns to 0, open the steam valve. Steam should flow out completely and then remove the sterilized objects. If steam is not released after returning the cursor to 0, water can condense and moisten plugs and sterilizable material (***objects will lose their sterility***).

The autoclave is sterilized:

a) glass, metal, porcelain, linen, rubber and cork stoppers, rubber, cellulose, wood products, dressings (cotton wool, gauze);

b) saline, solutions for injection, eye drops, distilled water, simple nutrient media (MPB, MPA);

c) mineral, plant oils in hermetically sealed flask.

Dry heat sterilization (hot air oven) can also be used on many objects in the absence of water. The items to be sterilized are placed in an oven at 160 to 170°C for 2 to 3 hours**. Oxidation of cell constituents and denaturation of proteins results in the death of microbes**. Most laboratories sterilize glass Petri dishes and pipettes with dry heat. This method though is not suitable for heat sensitive materials like many plastic

Used also for objects that won't melt or go bad: heat-resistant powdered medicinal products (talc, white clay, zinc oxide, etc.), mineral and plant oils, fats, lanolin, petroleum jelly, wax.

The device **of hot air oven (Pasteur oven)** and the order of work. The Pasteur oven is a double-skinned metal cabinet having outside with a material that does not conduct heat well (asbestos). Oven has an automatic temperature controller that maintains the set temperature. Heated air circulates in the space between the walls and exits through special openings. In the upper wall of the cabinet is a hole for the thermometer, which indicates the temperature inside the cabinet. When working, you must strictly monitor the correct temperature and sterilization time. If the temperature is higher, cotton plugs, paper in which the dishes are wrapped will be burned and at a lower temperature will require a longer sterilization time. At the end of the sterilization, the cabinet is opened only after it has cooled down, otherwise glassware may become cracked due to a rapid change in temperature.

HOT AIR OVEN

Intermittent boiling. This method is used for **sterilization of the media with gelatin, vitamins, carbohydrates, for some drugs,** which are spoiled at temperatures above 100^0C.

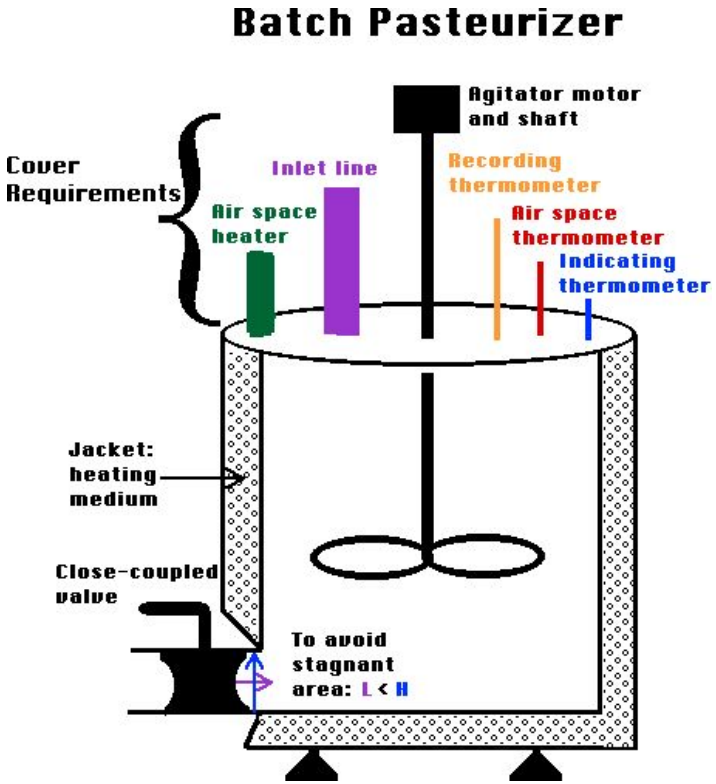
As after a single boiling (T=100 ° C) there is not killing of endospores, **intermittent boiling** is used: 20-30 min daily for 3 days. In the intervals between boiling, the material is kept at room temperature so that the endospores grow into vegetative forms, which will be killed with subsequent boiling.

Methods **of intermittent boiling** include **tindalization**. Tindalization is carried out in a water bath at 56 ° C for 1 hour 5-6 days. It is used to sterilize objects which are subjected to denaturation at a temperature of 100 ° C: serum, ascitic fluid, vitamins.

Methods of microbial control with heating also include **pasteurization.** It is carried out at a relatively low temperature once for the objects that lose their quality at high temperatures. The pasteurization does not refer to sterilization as endospores remain viable, so these products need to be stored in the cold (in a refrigerator).

Pasteurization is a process where many substances such as milk, are treated with heating at temperatures well below boiling (in honour of its developer Louis Pasteur). Milk, beer and many other beverages are now pasteurized. Pasteur examined the spoiled wine and detected the presence of microorganisms like bacteria which were responsible for the production of lactic acid and acetic acid fermentations which resulted in the spoilage of wine. He then discovered that brief heating at 55 to 60°C would destroy these microbes and preserve wine for long periods. Hence, pasteurization does not sterilize a beverage or milk but kills any pathogens present and slows spoilage by reducing the level of non-pathogenic spoilage microbes.

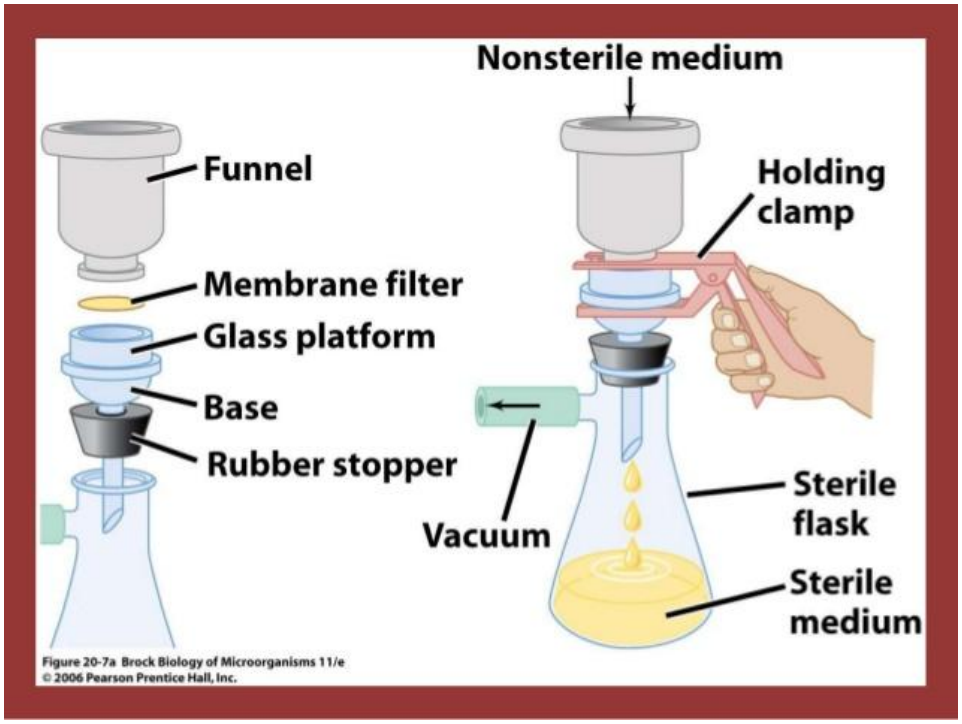
Milk in older methods of pasteurization (batch method) was held at 63°C for 30 min. Kills most vegetative bacterial cells including pathogens such as streptococci, staphylococci and *Mycobacterium tuberculosis*



Now, mostly two methods are used, **flash pasteurization** or high temperature short-term (HTST) pasteurization, which consists of quick heating t about 72°C for 15 sec and then rapid cooling. The other method used in dairy industry is **ultrahigh-temperature** (UHT) sterilization, where milk and milk products are heated at 140 to 150°C for 1 to 3 sec. The products pasteurized by this method needs no refrigeration and can be stored at room temperature for about 2 months.

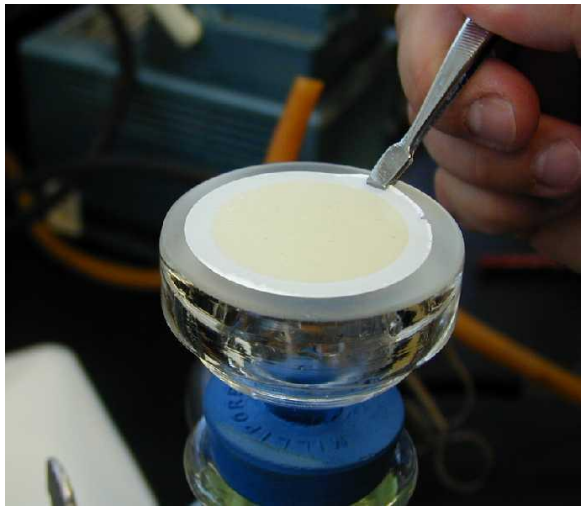
FILTRATION

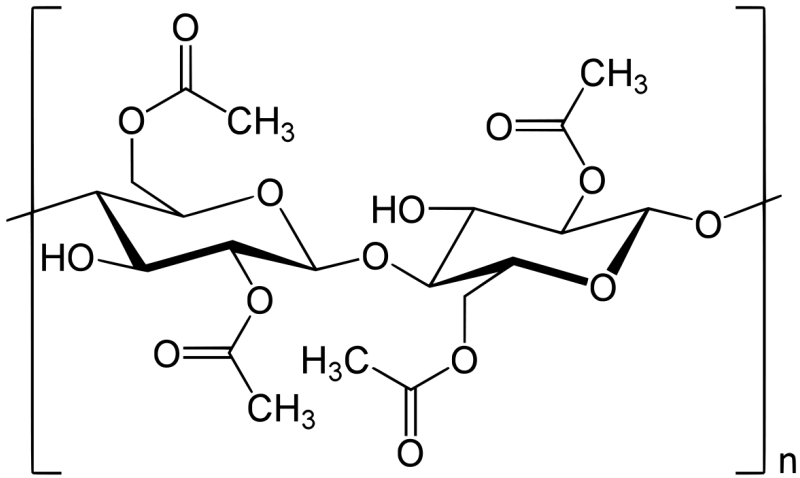
In order to sterilize solutions which is heat sensitive, filtration is an excellent way to reduce the microbial population. The filters simply remove the microbes instead of killing them. Depth filters consists of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter. The solution is passed through the filter which is sucked through this layer under vacuum and microbial cells are removed.



FILTRATION

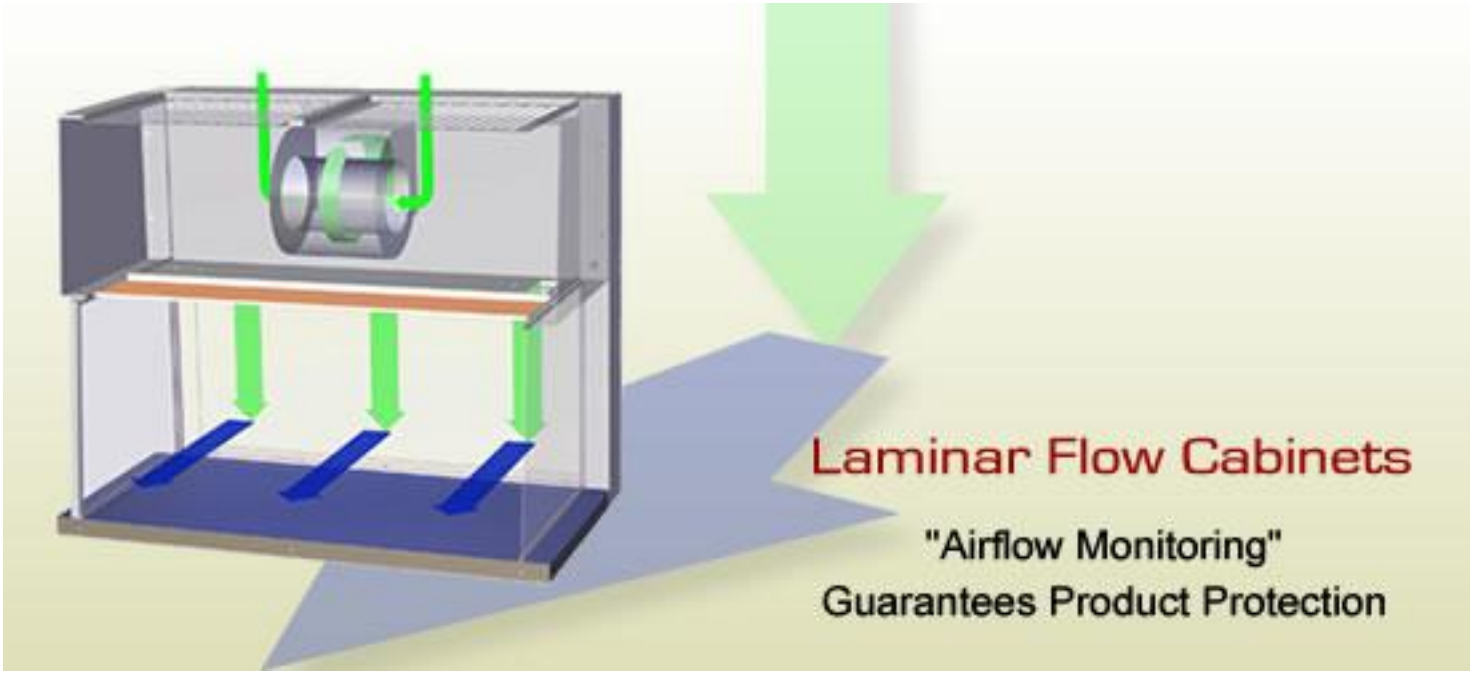
The material used mostly is unglazed porcelain, asbestos or other similar materials. Membrane filters are also used and have replaced depth filters in recent times. These filters are made up of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride, and other synthetic materials. These filters vary in size with pore sizes mostly of 0.2 to 0.5 µm in diameter and used to remove most vegetative cells, but not viruses, from solutions ranging in volume from 1ml to many litres. These filters are mostly used **to sterilize pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics and other heat sensitive solutions.**

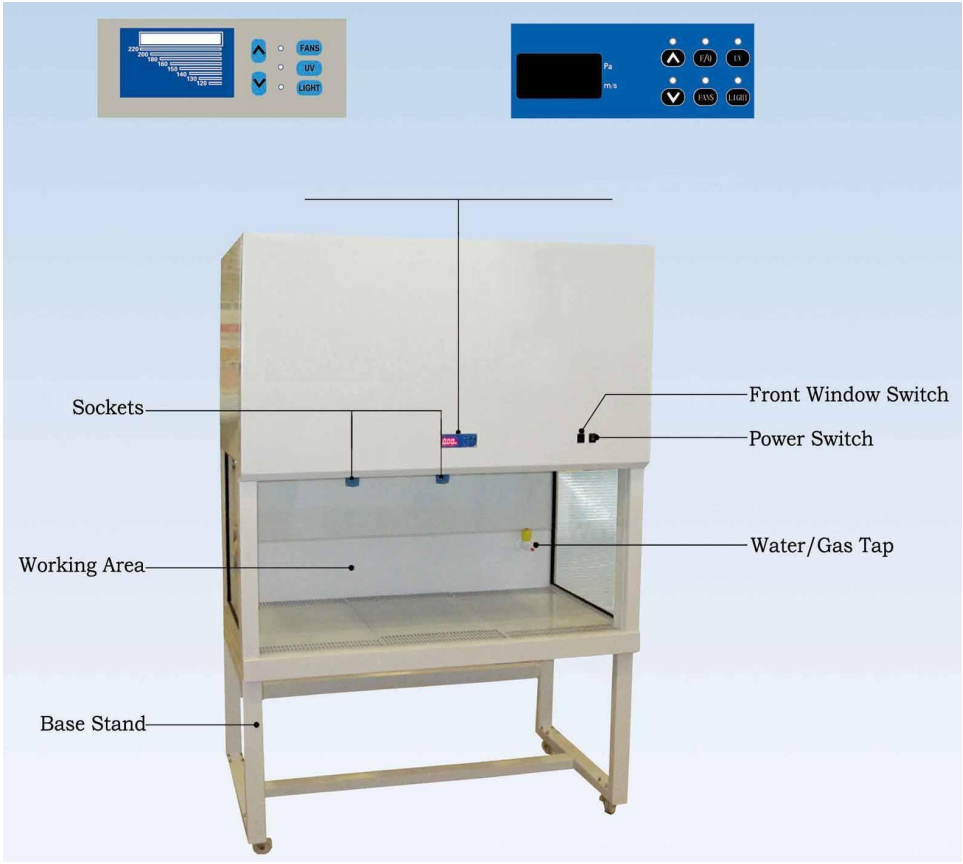




cellulose acetate

The other way this method is used is in the **laminar flow biological safety cabinets** where the air is sterilized by filtration. These cabinets contain high-efficiency particulate air (HEPA) filters, which remove 99.97% of 0.3µm particles. The safety cabinets are most useful as the culturing of any organisms requires contamination free air to reduce the growth of other undesired organisms or for the preparation of media, examining tissue cultures etc (Fig. 10).

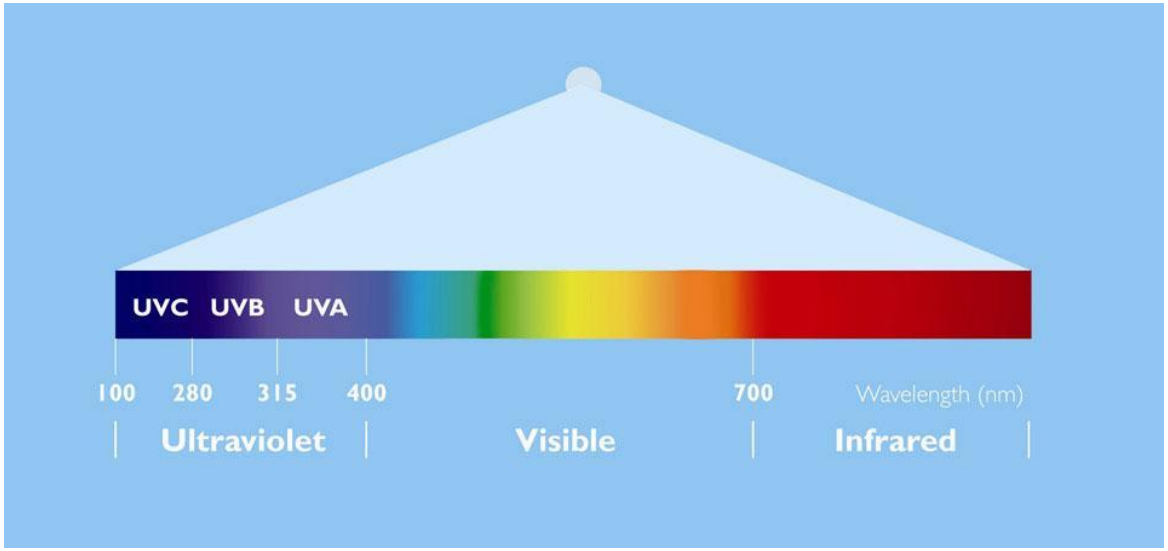




Laminar Flow Biological Safety Cabinets

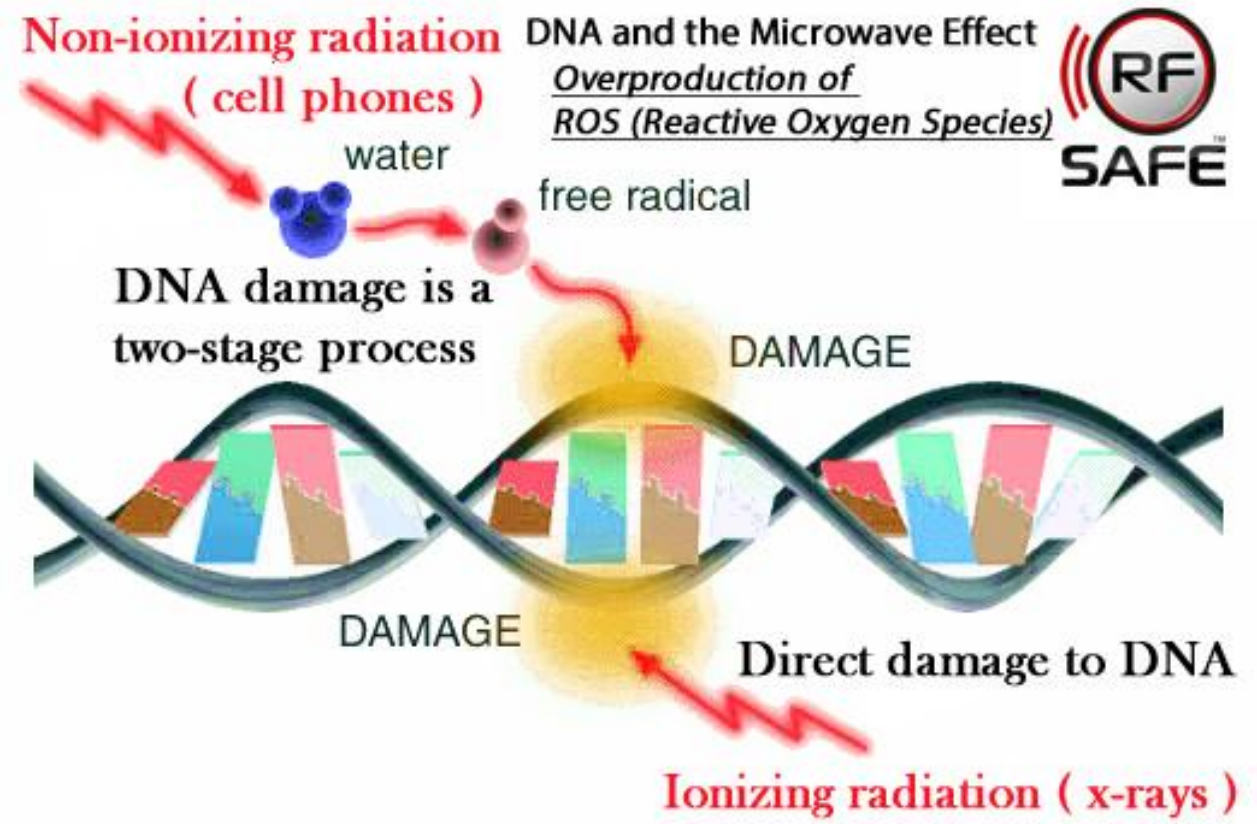
RADIATION

We have discussed about the effects of radiation on the growth of microorganisms earlier. The radiations like ultraviolet and ionizing can be used for sterilizing objects. **Ultraviolet radiation** around 260 nm is quite lethal but does not penetrate glass, dirt films, water and other substances very effectively.

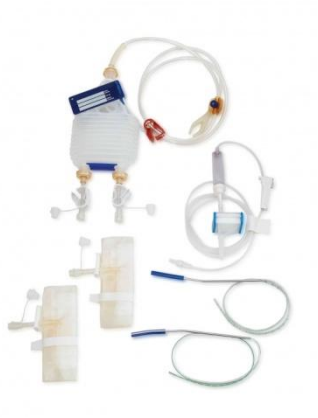


UV radiation is used as a sterilizing agent only in a few specific situations, like UV lamps are placed on the ceilings of rooms or in biological safety cabinets to sterilize air and other exposed surfaces. Commercial UV units are available for water treatment. Pathogens and microorganisms are destroyed when a thin layer of water is passed under the lamps (water purifiers). **Ultraviolet radiation** are safe to the operator of sterilization, they can be used even at the door entrances to prevent entry of live microbes through the air.

Ionizing radiation penetrates deep into objects and is an excellent sterilizing agent. It destroys bacterial endospores and vegetative cells of both prokaryotic and eukaryotic origin but not against viruses.



Gamma radiation from a cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures and plastic disposable supplies such as syringes, and Petri dishes, dressings, blood transfusion systems. Used for sterilization of objects that are not resistent for thermal and chemical treatment methods. It does not change the quality of the product, does not cause denaturation of the constituent parts of the product.



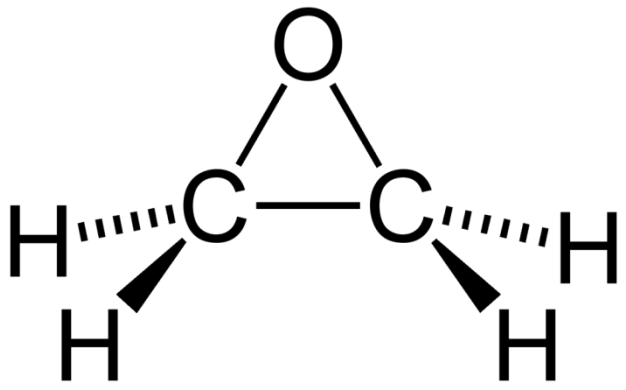
Also, ultrasonic are being tested for sterilization. Though it is not as effective as other methods, it was found to be useful in tissue cultures. Here the aim is to sterilize or even prevent the growth of bacteria during culturing of tissue. For ultrasonic sterilization, special ultrasonic transducers are used. Sterilize food products (their nutritional value is kept as much as possible), vaccines and some objects of laboratory equipment that spoil under the influence of high temperature and chemical sterilization

The Chemical Methods of Sterilization

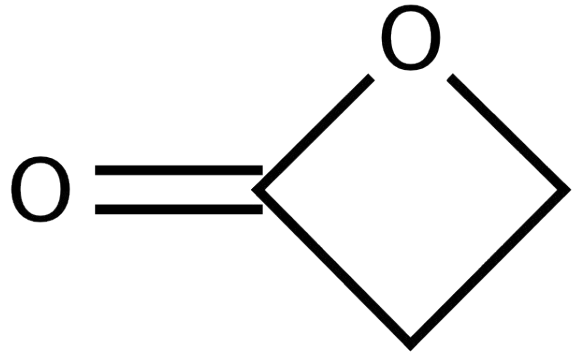
1. Sterilizing gases:

Gases may also be used as sterilizing agents in order to sterilize many heat-sensitive items such as disposable Petri dishes and many syringes, heart-lung machine components, sutures, Pacemakers, optics etc.

Ethylene oxide gas is used for this purpose as it readily penetrates packing materials, even plastic wraps and is both microbicidal and sporicidal and kills by combining with cell proteins. Betapropiolactone (BPL) is occasionally used as a sterilizing gas in the liquid form to sterilize vaccines and sera. The gasses used for sterilization are very poisonous. After sterilization, the gas is removed by blowing sterile air. It is mandatory to control the residual concentration of gases in the material.

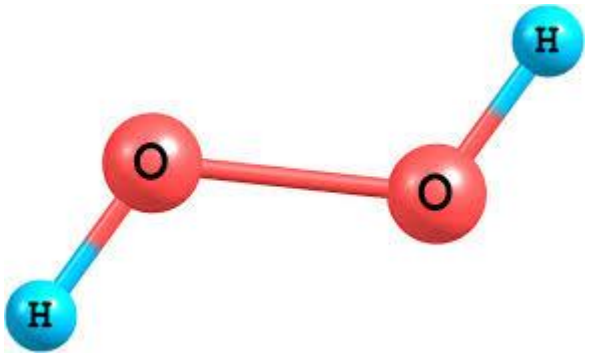


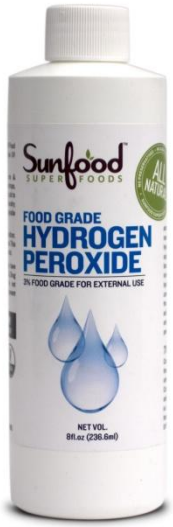
Ethylene oxide



Betapropiolactone

Recently vapour-phase hydrogen peroxide has been used to decontaminate biological safety cabinets. To do this, use a 3% solution of hydrogen peroxide and a 0, 5% solution of lactic acid. These solutions are sprayed 40-50 minutes before operation. As a result, air contamination decreases 30-40 times. Hydrogen peroxide is also used for the treatment of various surfaces: 3% hydrogen peroxide solution - for daily cleaning of industrial premises and 6% solution - for general cleaning.





Sterilization Monitoring by Physical, Chemical and Biological Indicators

How is the sterilization process monitored?

Sterilization procedures should be monitored through a combination of mechanical, chemical, and biological techniques designed to evaluate the sterilizing conditions and the procedure's effectiveness.

Mechanical techniques for monitoring sterilization include assessing the cycle time, temperature, and pressure of sterilization equipment by observing the gauges or displays on the sterilizer. Some tabletop sterilizers have recording devices that print out these parameters. Correct readings do not ensure sterilization, but incorrect readings could be the first indication that a problem has occurred with the sterilization cycle.

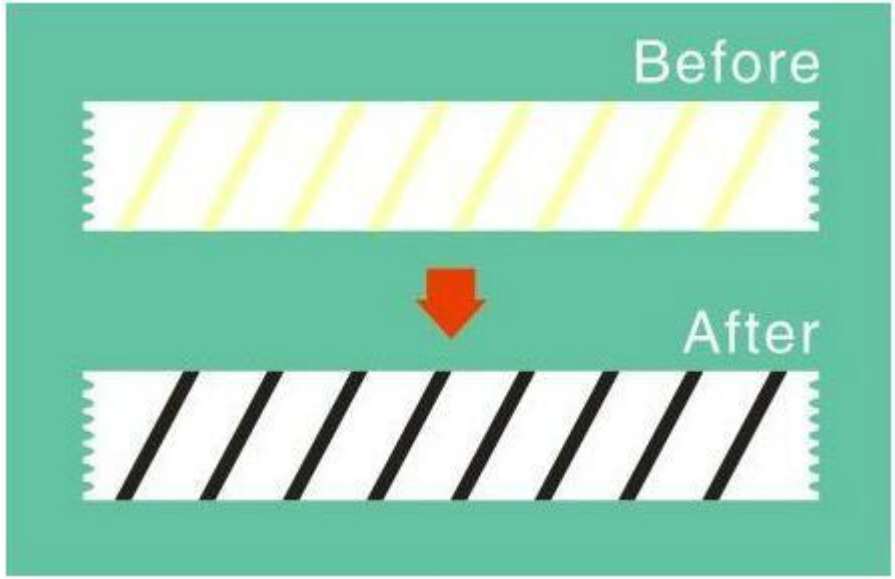
- Chemical indicators, internal and external, use sensitive chemicals to assess **physical conditions such as temperature** during the sterilization process.

Chemical Color Change Indicators – are powder substances with a strictly defined melting point: benzonaphthol (110 ° C), antipyrine (113 ° C), resorcinol and sulfur (119 ° C), benzoic acid (120 ° C). These substances are mixed with a small amount of dry aniline paints (magenta, methylene blue) and placed in sealed glass tubes between sterilized objects. Chemical indicators should be positioned near the center of each load, and toward the bottom front of the autoclave.

If the temperature in the autoclave was sufficient, the substance in the tube melts and stains the color of the dye that dissolves in this substance. Hence, chemical indicators can give a quick visual reference for heat penetration inside the autoclave.

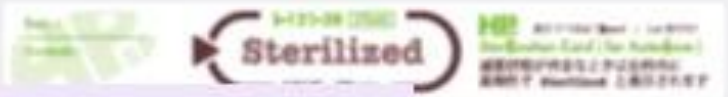
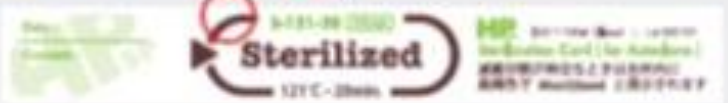
Tape Indicators

Tape indicators are adhesive backed paper tape with heat sensitive, chemical indicator markings. Commonly used heat sensitive markings include diagonal stripes (autoclave tape), and/or the word “sterile”. These markings only appear when the tape has been exposed for a few minutes to normal autoclave decontamination temperatures.



Example of color change

TEMP: 121 deg.C

S-121-20	AC-008
	
Before sterilization	Before sterilization
	
5 min	5 min
	
10 min	10 min
Reference color after color change is completed. 	
20 min	20 min

ISO 11140 Class4

Tape Indicators

An internal tape indicators should be placed in every sterilization package to ensure the sterilization agent has penetrated the packaging material and actually reached the instruments inside. An external indicator should be used when the internal indicator cannot be seen from outside the package.

Caution: Most chemical indicators and tape indicators can only be used to verify that your autoclave has reached normal operating temperatures for decontamination; they have no time factor. Chemical indicators alone are not designed to prove that organisms are actually killed during a decontamination cycle.

Indicator test results are shown immediately after the sterilization cycle is complete and could provide an early indication of a problem and where the problem occurred in the process. If the internal or external indicator suggests inadequate processing, the item that has been processed should not be used. Because chemical indicators do not prove sterilization has been achieved, a biological indicator (i.e., spore test) is required.

BIOLOGICAL INDICATORS

Biological indicators (BIs) are the most accepted means **of monitoring the sterilization** process because they directly determine whether the most resistant microorganisms (e.g., Geobacillus or Bacillus species) are present rather than merely determine whether the physical and chemical conditions necessary for sterilization are met. Because spores used in BIs are more resistant and present in greater numbers than are the common microbial contaminants found on patient care equipment, an inactivated BI indicates that other potential pathogens in the load have also been killed.

Biological indicators are designed to demonstrate that an autoclave is capable of killing microorganisms. EH&S recommends the use of commercially available *Geobacillus stearothermophilus* spores to monitor the effectiveness of steam autoclaves. This test must be performed at least every 90 days.

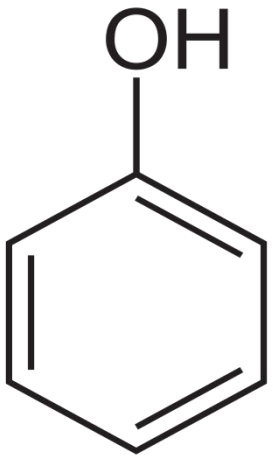
Geoacillus stearothermophilus spores die at 121 ° C for 15 minutes when they are contained in 1 ml of a medium of 10^6 cells. Tubes with strips of gauze, filter paper, with silk thread, infected with spores, are placed between sterilizable objects. After sterilization, a nutrient broth (MPB) is added into the test tube and the growth of microorganisms is observed. The presence of turbidity is a sign of bacterial grows, hence the autoclave is not capable of killing microorganisms and their spores and vice versa, if nutrient medium remains transparent - the autoclave is capable of killing microorganisms.

Use of Chemical Agents in Microbiological Control

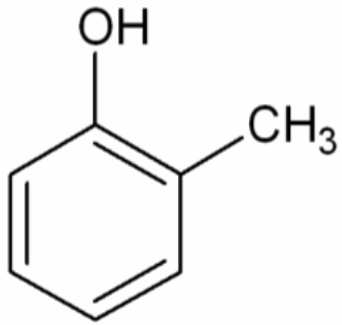
The chemical agents are mostly employed in **disinfection and antisepsis**. The proper use of these agents is essential to laboratory and hospital safety. Factors such as the kinds of microorganisms potentially present the concentration and nature of the **disinfectant** to be used and the length of treatment should be considered. Many disinfectants are available and each has its own advantages and disadvantages, but ideally the disinfectant must be effective against a wide variety of infectious agents, at high dilutions and in the presence of organic matter and should not be toxic to people or corrosive for common materials. The disinfectant must be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms, and have a low surface tension so that it can enter cracks in surfaces.

Phenols

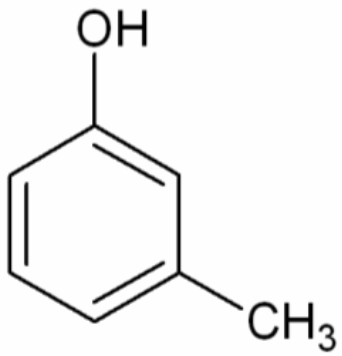
In 1867 Joseph Lister employed it to reduce the risk of infection during operations and phenol was the first widely used antiseptic and disinfectant. Today phenol and phenolics such as cresols, xylenols, are used as disinfectants in laboratories and hospitals.



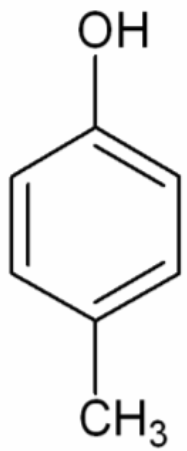
phenol



o-cresol



m-cresol



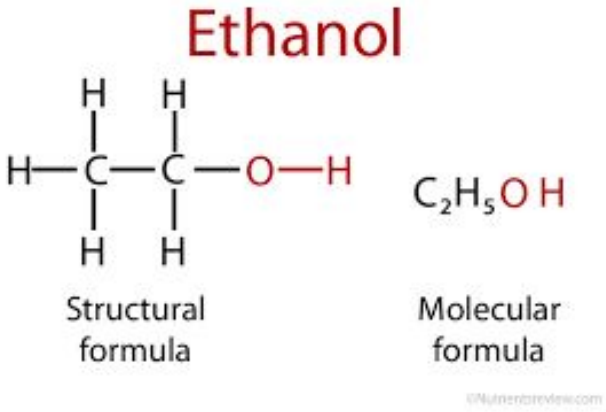
p-cresol

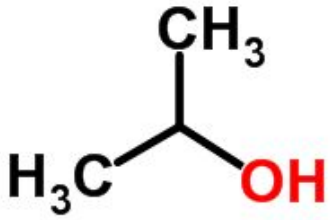
Phenols

Lysol is made of a mixture of phenolics which is commercially available **disinfectant.** They act by denaturing proteins and disrupting cell membranes. Phenolics are tuberculocidal and effective in the presence of organic material and remain active on surfaces long after application. However, they do have a disagreeable odour and can cause skin irritation. **Hexachlorophene** has been one of the most popular **antiseptics** because once applied it persists on the skin and reduces skin bacteria for long periods.

ALCOHOLS

Alcohols are the most widely used **disinfectants and antiseptics**. They are bactericidal and fungicidal but not sporicidal. Ethanol and isopropanol are the two most popular alcohol germicides. They act by denaturing proteins and possibly by dissolving membrane lipids. Small instruments like thermometers can be disinfected by soaking them for 10 to 15 min in alcohol solutions. A 70% ethanol is more effective than 95% as water is needed for proteins to coagulate.





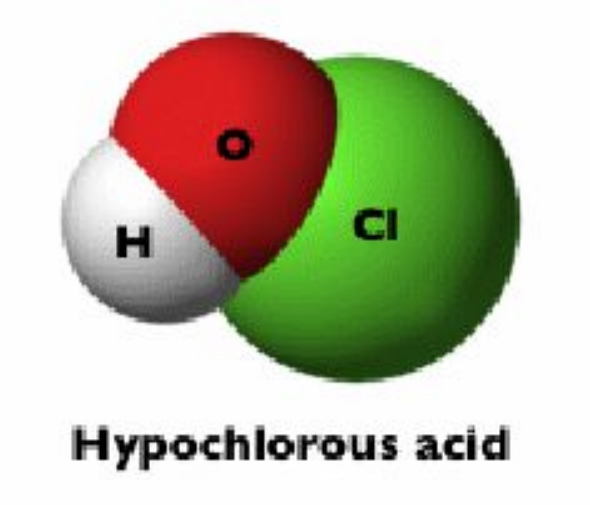
HALOGENS

Halogens exist as diatomic molecules in the free state and form salt like compounds with sodium and most other metals. **Iodine and chlorine** are the most important antimicrobial agents. **Iodine is used as a skin antiseptic** and kills by oxidizing cell constituents and iodinating cell proteins. Spores can be destroyed at higher concentrations.

HALOGENS

Iodine is often applied as tincture of iodine, 2% or more iodine in a water-ethanol solution of potassium iodide. Skin scars result and sometimes iodine allergies can result. In today's date, brands like Wescodyne for skin and laboratory disinfection and for wounds is being used as iodine is complexed with an organic carrier to form iodophor; and these are mostly used in hospitals for preoperative skin degerming and in hospitals and laboratories for **disinfection.**

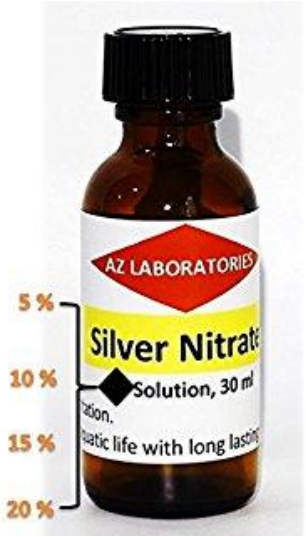
Chlorine is mostly used **as a disinfectant** for municipal water supplies and swimming pools and also employed in dairy and food industry. It may be applied as chlorine gas, sodium hypochloride or calcium hypochloride, all of which yield hypochlorous acid (HClO) and then atomic oxygen. The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores. One potential problem is that chlorine reacts with organic compounds to form carcinogenic trihalomethanes, which must be monitored in drinking water. Ozone sometimes has been used successfully as an alternative to chlorination in Europe and Canada. Small amounts of drinking water can be disinfected with halazone tablets. It slowly releases chloride when added to water and disinfects it in about half an hour.

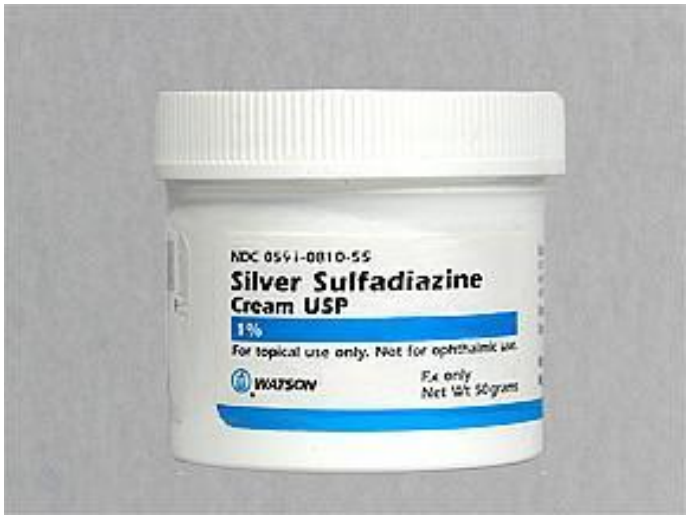


Hypochlorous acid

HEAVY METALS

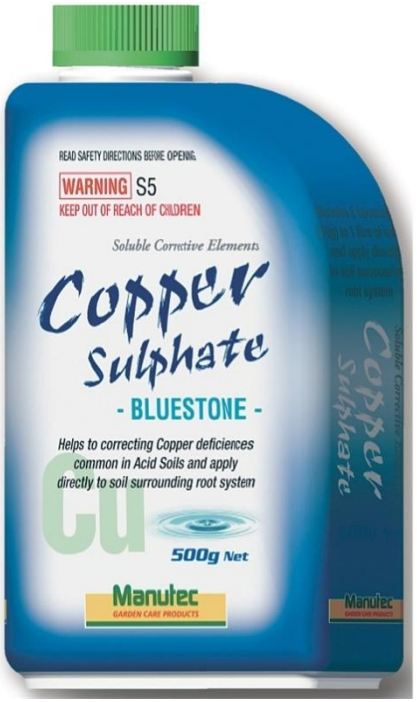
Heavy metals such as mercury, silver, arsenic, zinc and copper were used as germicides and these have been most recently superseded by other less toxic and more effective germicides. A 1% solution of silver nitrate is often added to the eyes of infants to prevent ophthalmic gonorrhea but now erythromycin is used instead of silver nitrate because it is effective against *Chlamydia* as well as *Neisseria.* Silver sulfadiazine is used on burns.





HEAVY METALS

Copper sulphate is an effective algicide in lakes and swimming pools. The action of these heavy metals is mostly on the proteins, and they combine often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins.

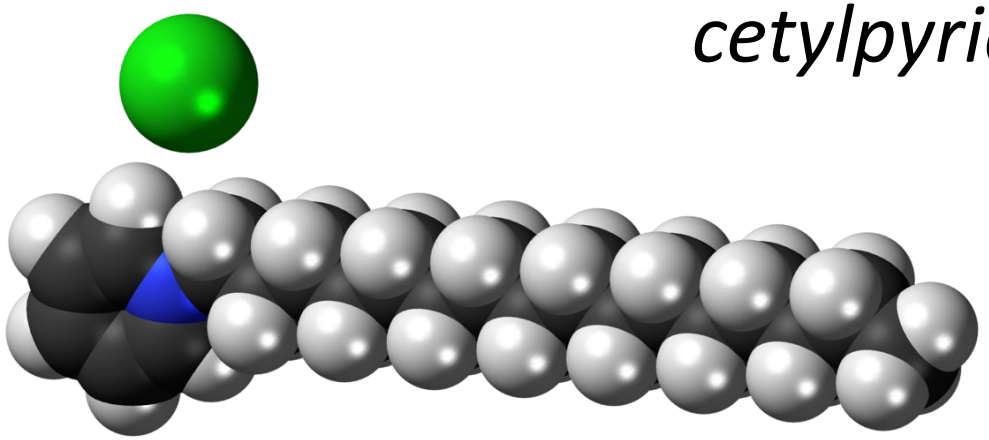


Quaternary Ammonium Compounds

Detergents are organic molecules that serve as wetting agents and emulsifiers and are amphipathic in nature and hence solubilize otherwise insoluble residues and are very effective cleansing agents and are different from soaps, which are derived from fats.

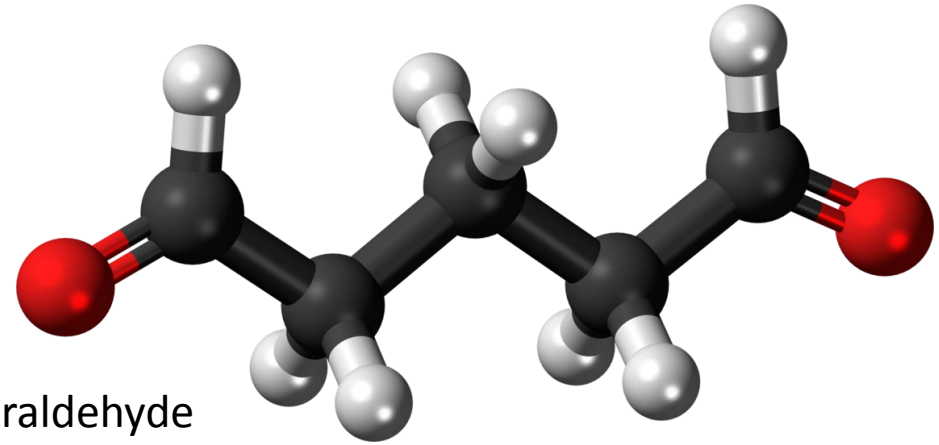
Only cationic detergents are effective **disinfectants** characterized by positively charged **quaternary nitrogen** and a long hydrophobic aliphatic chain. They disrupt microbial membranes and may also denature proteins. Mostly used as disinfectants for food utensils and small instruments and **as skin antiseptics**: **benzalkonium chloride and cetylpyridinium chloride**.

cetylpyridinium chloride



ALDEHYDES

Formaldehyde and glutaraldehyde are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by cross-linking and alkylating molecules. Formaldehyde is usually dissolved in water or alcohol before use. A 2% buffered solution of glutaraldehyde is **an effective disinfectant** and is mostly used to disinfect hospital and laboratory equipments. These are mostly sporicidal and can be used **as chemical sterilants**.



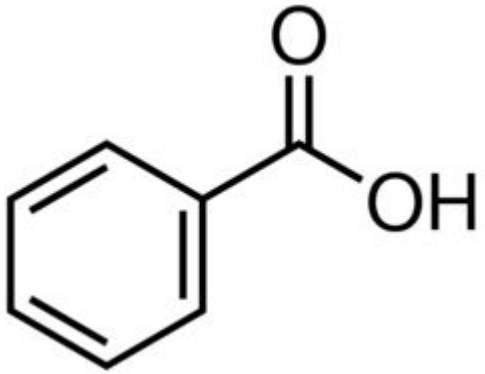
glutaraldehyde

HYDROGEN PEROXIDE

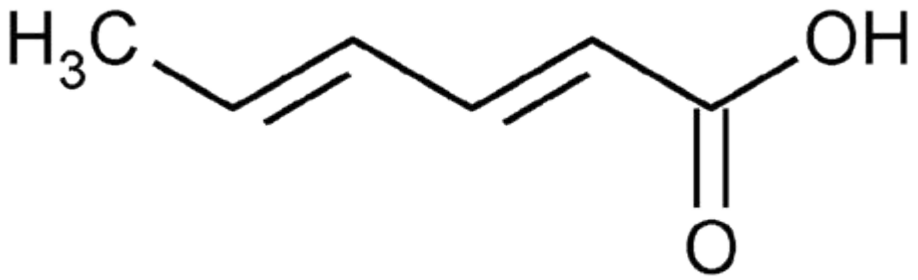
H_2O_2 effects are direct and indirect actions of O_2 as it forms hydroxyl free radical which is highly toxic and reactive to cell. **As an antiseptic**, 3% H_2O_2 serves a variety of needs including skin and wound cleansing, bedsore care and mouth washing. It is especially useful in treating infection by anaerobic bacteria because of the lethal effects of O_2 on these forms. When it is applied to a wound, the enzyme catalase in the tissue decomposes the H_2O_2 into water and free O_2. The O_2 causes the wound tissues to bubble and the bubbling removes microorganisms mechanically. Also, the sudden release of O_2 brings about chemical changes in certain microorganisms, and these changes lead to microbial death.

ACIDS AND ALKALIS

Conditions of very low or high pH can destroy or inhibit microbial cells; but they are limited in application due to their corrosive, caustic and hazardous nature. Aqueous solutions of ammonium hydroxide remain a common component of detergents, cleansers and deodorizers. Organic acids are widely used in food **preservation** because they prevent spore germination and bacterial and fungal growth. Acetic acid (in the form of vinegar) is a pickling agent that inhibits bacterial growth; propionic acid is commonly incorporated into breads and cakes to retard moulds, benzoic acid and sorbic acids are added to beverages, syrups etc to inhibit yeasts.



benzoic acid



sorbic acid

METHODS OF DISINFECTION

1. Physical:

a) mechanical (wet cleaning, washing, shaking out, airing)

b) the effect of temperature:

high (ironing, dry and moist hot air, calcination, boiling, burning),

and low (freeze);

c) radiation and ultrasonic.

2. Chemical - treatment of the object with disinfectants.

3. Biological (biological filters, composting).

4. Combined (combination of different methods).

Types of antisepsis:

- **mechanical (**removal from the wound of infected and non-viable tissues);

-**physical** (hygroscopic dressings, hypertensive solutions, UV, laser);

- **chemical** (application of chemical substances with antimicrobial action: miramistin, chlorhexidine, alcohol 70%, brilliant green, hydrogen peroxide, alcohol solution of iodine);

-**biological** (use of antibiotics, bacteriophages, etc.).

Aseptic - a system of preventive measures, a set of measures to prevent the entry of microorganisms from the environment into the tissue (wound), the body cavity under medical and diagnostic manipulations, into sterile medicinal drugs during their manufacture, into research material, nutrient media, microorganism cultures in laboratory studies.

For this purpose, in bacteriological laboratories, make inoculations at the flame of an alcohol lamp, previously ignited bacteriological loop, sterile nutrient media are used for cultivation. Aseptic is achieved by sterilization of surgical instruments and materials, treatment of the hands of the surgeon before the operation, air and objects of the operating room, the surface of the skin of the operating field, observance of certain rules (sterile coat, gloves, mask, exclusion of conversations), by wet cleaning of premises with disinfectants, use of bactericidal lamps etc.

PRESERVATIVES

1. Aldehydes (formaldehyde)
2. Guanidine derivatives (chlorhexidine derivatives)
3. Inorganic acids and their salts (boric acid, sodium sulfite)
4. Organic acids, their salts (benzoic acid, salicylic acid, sorbic acid)
5. Mercury compounds (merthiolate, phenylmercury nitrate).

The requirements for preservatives.

1) a broad spectrum of antimicrobial activity;
2) rapidity of biocidal action;
3) do not interact with medicinal substances;
4) stability;
5) are pharmacologically indifferent;
6) maintain the sterility of the drug throughout the life of the product.