

## **CONTROL MEASURES FOR THE GROWTH OF MICROORGANISMS**

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Control of microorganisms is essential in order to prevent the transmission of diseases and infection, stop decomposition and spoilage and prevent unwanted microbial contamination.

Microorganisms are controlled by means of physical agents and chemical agents.

1. Physical control includes such methods of control as high or low temperature, desiccation, osmotic pressure, radiation, and filtration.
2. Chemical control refers to the use of disinfectants, antiseptics, antibiotics, and chemotherapeutic antimicrobial chemicals.
3. Sterilization is the process of destroying all living organisms and viruses. A sterile object is one free of all life forms, including bacterial endospores, as well as viruses.
4. Disinfection is the elimination of microorganisms, but not necessarily endospores, from inanimate objects or surfaces.
5. Decontamination is the treatment of an object or inanimate surface to make it safe to handle.
6. A disinfectant is an agent used to disinfect inanimate objects but generally too toxic to use on human tissues.
7. An antiseptic is an agent that kills or inhibits growth of microbes but is safe to use on human tissue.
8. A sanitizer is an agent that reduces microbial numbers to a safe level.
9. An antibiotic is a metabolic product produced by one microorganism that inhibits or kills other microorganisms.
10. Synthetic chemicals that can be used therapeutically.
11. An agent that is cidal in action kills microorganisms.
12. An agent that is static in action inhibits the growth of microorganisms.
13. Selective toxicity means that the chemical being used should inhibit or kill the intended pathogen without seriously harming the host.

14. A broad spectrum agent is one generally effective against a variety of Gram-positive and Gram-negative bacteria.
15. A narrow spectrum agent generally works against just Gram-positives, Gram-negatives, or only a few bacteria.

**Keep in mind that when evaluating or choosing a method of controlling microorganisms, you must consider the following factors which may influence antimicrobial activity:**

1. The concentration and kind of a chemical agent used
2. The intensity and nature of a physical agent used
3. The length of exposure to the agent
4. The temperature at which the agent is used
5. The number of microorganisms present
6. The organism itself
7. The nature of the material bearing the microorganism.

#### **DETAILED EXPLANATION FOR CONTROL MEASURES OF MICROORGANISMS:**

**TEMPERATURE:** Generally, an increase in temperature will increase enzyme activity. But if temperatures get too high, enzyme activity will diminish and the protein (the enzyme) will denature.

On the other hand, lowering temperature will decrease enzyme activity. At freezing temperatures enzyme activity can stop. Repeated cycles of freezing and thawing can denature proteins. In addition, freezing causes water to expand and also forms ice crystals, hence cells begin to rupture.

Every bacterial species has specific growth temperature requirements which is largely determined by the temperature requirements of its enzymes. Each organism will have:

- MINIMUM GROWTH TEMPERATURE
- OPTIMUM GROWTH TEMPERATURE

- MAXIMUM GROWTH TEMPERATURE

Organisms can be classified according to their optimum growth temperature.:

- PSYCHROPHILES grow best between  $-5^{\circ}\text{C}$  and  $20^{\circ}\text{C}$ ,
- MESOPHILES grow best between  $20^{\circ}\text{C}$  and  $45^{\circ}\text{C}$  and
- THERMOPHILES grow best at temperatures above  $45^{\circ}\text{C}$ .
- THERMODURIC organisms can survive high temperatures but don't grow well at such temperatures. Organisms which form endospores would be considered thermoduric.

Some organisms have exotic temperature requirements. *Thermus aquaticus* is a bright orange gram negative rod isolated from hot water and steam vents at Yellowstone Park. This organism grows best at temperatures between  $70-75^{\circ}\text{C}$  ( $158-167^{\circ}\text{F}$ ). Some of its unique enzymes are in demand for molecular biological and industrial applications.

Microorganisms have a minimum, an optimum, and a maximum temperature for growth. Temperatures below the minimum usually have a static action on microorganisms. They inhibit microbial growth by slowing down metabolism but do not necessarily kill the organism. Temperatures above the maximum usually have a cidal action, since they denature microbial enzymes and other proteins. Temperature is a very common and effective way of controlling microorganisms.

**High Temperature:** Vegetative microorganisms can generally be killed at temperatures from  $50^{\circ}\text{C}$  to  $70^{\circ}\text{C}$  with moist heat. Bacterial endospores, however, are very resistant to heat and extended exposure to much higher temperature is necessary for their destruction. High temperature may be applied as either moist heat or dry heat.

**Moist heat:** Moist heat is generally more effective than dry heat for killing microorganisms because of its ability to penetrate microbial cells. Moist heat kills microorganisms by denaturing their proteins (causes proteins and enzymes to lose their three-dimensional functional shape). It also may melt lipids in cytoplasmic membranes.

**Autoclaving:** Autoclaving employs steam under pressure. Water normally boils at 100°C; however, when put under pressure, water boils at a higher temperature. During autoclaving, the materials to be sterilized are placed under 15 pounds per square inch of pressure in a pressure-cooker type of apparatus. When placed under 15 pounds of pressure, the boiling point of water is raised to 121°C, a temperature sufficient to kill bacterial endospores. The time the material is left in the autoclave varies with the nature and amount of material being sterilized. Given sufficient time (generally 15-45 minutes), autoclaving is lethal for both vegetative organisms and endospores, and is the most common method of sterilization for materials not damaged by heat.

**Boiling water:** Boiling water (100°C) will generally kill vegetative cells after about 10 minutes of exposure. However, certain viruses, such as the hepatitis viruses, may survive exposure to boiling water for up to 30 minutes, and endospores of certain *Clostridium* and *Bacillus* species may survive even hours of boiling.

**Dry heat:** Dry heat kills microorganisms through a process of protein oxidation rather than protein coagulation.

**Hot air sterilization:** Microbiological ovens employ very high dry temperatures: 171°C for 1 hour; 160°C for 2 hours or longer; or 121°C for 16 hours or longer depending on the volume. They are generally used only for sterilizing glassware, metal instruments, and other inert materials like oils and powders that are not damaged by excessive temperature.

**Pasteurization:** Pasteurization is the mild heating of milk and other materials to kill particular spoilage organisms or pathogens. It does not, however, kill all organisms. Milk is usually pasteurized by heating to 71.6°C for at least 15 seconds in the flash method or 62.9°C for 30 minutes in the holding method.

**Low Temperature:** Low temperature inhibits microbial growth by slowing down microbial metabolism. Examples include refrigeration and freezing. Refrigeration at 5°C slows the growth of microorganisms and keeps food fresh for a few days. Freezing at -10°C stops microbial growth, but generally does not kill microorganisms, and keeps food fresh for several months.

**DESICCATION:** Desiccation, or drying, generally has a static effect on microorganisms. Lack of water inhibits the action of microbial enzymes. Dehydrated and freeze-dried foods, for example, do not require refrigeration because the absence of water inhibits microbial growth.

**OSMOTIC PRESSURE:** The canning of jams or preserves with a high sugar concentration inhibits bacterial growth through hypertonicity. The same effect is obtained by salt-curing meats or placing foods in a salt brine. This static action of osmotic pressure thus prevents bacterial decomposition of the food. Molds, on the other hand, are more tolerant of hypertonicity. Foods, such as those mentioned 4 above, tend to become overgrown with molds unless they are first sealed to exclude oxygen. (Molds are aerobic.)

**Ultraviolet Radiation:** UV lights are frequently used to reduce the microbial populations in hospital operating rooms and sinks, aseptic filling rooms of pharmaceutical companies, in microbiological hoods, and in the processing equipment used by the food and dairy industries. An important consideration when using UV light is that it has very poor penetrating power. Only microorganisms on the surface of a material that are exposed directly to the radiation are susceptible to destruction. UV light can also damage the eyes, cause burns, and cause mutation in cells of the skin.

**FILTRATION:** Microbiological membrane filters provide a useful way of sterilizing materials such as vaccines, antibiotic solutions, animal sera, enzyme solutions, vitamin solutions, and other solutions that may be damaged or denatured by high temperatures or chemical agents. The filters contain pores small enough to prevent the passage of microbes but large enough to allow the organism free fluid to pass through. The liquid is then collected in a sterile flask.

**Phenol and phenol derivatives:** Phenol (5-10%) was the first disinfectant commonly used. However, because of its toxicity and odor, phenol derivatives are now generally used. These include orthophenylphenol, hexachlorophene, triclosan, hexylresorcinol, and chlorhexidine. Orthophenylphenol is the agent in Lysol, O-syl, Staphene and Amphyl. Hexachlorophene in a 3% solution is combined with detergent and is found in PhisoHex. Triclosan is a chlorine-containing phenolic antiseptic very common in antimicrobial soaps and other products.

Hexylresorcinol is in throat lozenges and ST-37. A 4% solution of chlorhexidine in isopropyl alcohol and combined with detergent (Hibiclens and Hibitane) is a common hand washing agent and surgical hand scrub. These agents kill most bacteria, most fungi, and some viruses, but are usually ineffective against endospores. They alter membrane permeability and denature proteins.

**Soaps:** They are only mildly microbicidal. Their use aids in the mechanical removal of microorganisms by breaking up the oily film on the skin (emulsification) and reducing the surface tension of water so it spreads and penetrates more readily. Some cosmetic soaps contain added antiseptics to increase antimicrobial activity.

**Alcohol:** 70% solutions of ethyl or isopropyl alcohol are effective in killing vegetative bacteria, enveloped viruses, and fungi. However, they are usually ineffective against endospores and non-enveloped viruses. Once they evaporate, their cidal activity will cease. Alcohols denature membranes and are often combined with other disinfectants, such as iodine, mercurial and cationic detergents for increased effectiveness.

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