Control of Microorganisms

Microbiology

- Although many microorganisms are beneficial and necessary for human well-being, microbial activities may have undesirable consequences such as food spoilage and disease.
- Therefore it is essential to be able to kill a wide variety of microorganisms or inhibit their growth to minimize their destructive effects.

The goal is two fold:

(1) to destroy pathogens and prevent their transmission,

(2) to reduce or eliminate microorganisms responsible for the contamination of water, food, and other substances.

Sterilization [Latin sterilis, unable to produce offspring or barren] is the process by which all living cells, viable spores, viruses, and Viroids are either destroyed or removed from an object or habitat.

- A sterile object is totally free of viable microorganisms, spores, and other infectious agents.
- When sterilization is achieved by a chemical agent, the chemical is called a sterilant.

Disinfection:

- It is the process of killing, inhibition, or removal of microorganisms that may cause disease.
- The primary goal is to destroy potential pathogens, but disinfection also substantially reduces the total microbial population.
- **Disinfectants** are agents, usually chemical, used to carry out disinfection and are normally used only on inanimate objects.
- A disinfectant does not necessarily sterilize an object because viable spores and a few microorganisms may remain.

Sanitization

- Sanitization is closely related to disinfection.
- In sanitization, the microbial population is reduced to levels that are considered safe by public health standards.
- The inanimate object is usually cleaned as well as partially disinfected. For example, sanitizers are used to clean eating utensils in restaurants.

Antisepsis

- Antisepsis [Greek anti, against, and sepsis, putrefaction] is the prevention of infection and is accomplished with antiseptics.
- **Antiseptics** are **chemical agents** applied to tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population.
- Because they must not destroy too much host tissue, antiseptics are generally not as toxic as disinfectants.

Germicide:

- Substances that kill organisms often have the suffix –cide [Latin cida, to kill]: a germicide kills pathogens (and many non pathogens) but not necessarily endospores.

- A disinfectant or antiseptic can be particularly effective against a specific group, in which case it may be called a **bactericide, fungicide, algicide, or viricide.**

- Other chemicals do not kill, but they do prevent growth. If these agents are removed, growth will resume. Their names end in -static [Greek statikos, causing to stand or stopping]—for example, **bacteriostatic and fungistatic.**

The Use of Physical Methods in Control

Heat

- Fire and boiling water have been used for sterilization and disinfection since the time of the Greeks, and heating is still one of the most popular ways to destroy microorganisms.
- Either moist or dry heat may be applied.
- Moist heat readily kills viruses, bacteria, and fungi. Exposure to boiling water for 10 minutes is sufficient to destroy vegetative cells and eukaryotic spores.
- Unfortunately the temperature of boiling water (100°C or 212°F) is not high enough to destroy bacterial endospores that may survive hours of boiling.
- Therefore boiling can be used for disinfection of drinking water and objects not harmed by water, but boiling does not sterilize.

Table 7.2 Approximate Conditions for Moist Heat Killing

Organism	Vegetative Cells	Spores
Yeasts	5 minutes at 50–60°C	5 minutes at 70–80°C
Molds	30 minutes at 62°C	30 minutes at 80°C
Bacteria[a]	10 minutes at 60–70°C	2 to over 800 minutes at 100°C 0.5–12 minutes at 121°C
Viruses	30 minutes at 60°C	

[a]Conditions for mesophilic bacteria.

Moist heat sterilization

- Moist heat sterilization must be carried out at temperatures above 100°C in order to destroy bacterial endospores, and this requires the use of saturated steam under pressure.

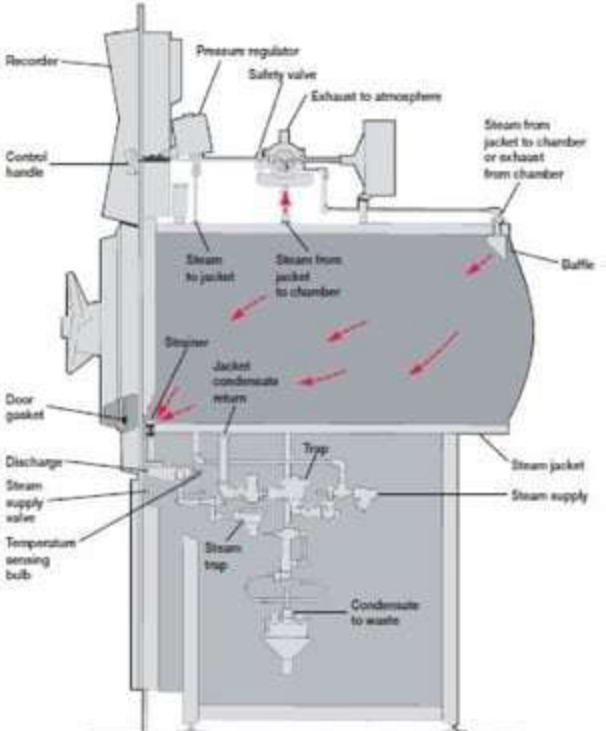
- Steam sterilization is carried out with an **autoclave.**

- The development of the autoclave by Chamberland in 1884 tremendously stimulated the growth of microbiology.

- Water is boiled to produce steam, which is released through the jacket and into the autoclave's chamber.
- The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed.
- Hot, saturated steam continues to enter until the chamber reaches the desired temperature and pressure, usually 121°C and 15 pounds of pressure.
- At this temperature saturated steam destroys all vegetative cells and endospores in a small volume of liquid within 10 to 12 minutes.
- Treatment is continued for about 15 minutes to provide a margin of safety.
- Of course, larger containers of liquid such as flasks and carboys will require much longer treatment times.
- 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.



(a)



(b)

Figure 7.3 The Autoclave or Steam Sterilizer. (a) A modern, automatically controlled autoclave or sterilizer. (b) Longitudinal cross section of a typical autoclave showing some of its parts and the pathway of steam. (b) *From John J. Perkins, Principles and Methods of Sterilization in Health Science, 2nd edition, 1969. Courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.*

- Autoclaving must be carried out properly or the processed materials will not be sterile.
- The chamber should not be packed too tightly because the steam needs to circulate freely and contact everything in the autoclave.
- Bacterial endospores will be killed only if they are kept at 121°C for 10 to 12 minutes.
- When a large volume of liquid must be sterilized, an extended sterilization time will be needed because it will take longer for the center of the liquid to reach 121°C; 5 liters of liquid may require about 70 minutes.

Pasteurization

- Many substances, such as milk, are treated with controlled heating at temperatures well below boiling, a process known as **pasteurization,** in honor of its developer Louis Pasteur.
- Pasteur examined spoiled wine under the microscope and detected microorganisms that looked like the bacteria responsible for lactic acid and acetic acid fermentations.
- He then discovered that a brief heating at 55 to 60°C would destroy these microorganisms and preserve wine for long periods.
- Milk pasteurization was introduced into the United States in 1889. Milk, beer, and many other beverages are now pasteurized.
- Pasteurization does not sterilize a beverage, but it does kill any pathogens present and drastically slows spoilage by reducing the level of nonpathogenic spoilage microorganisms.
- Milk can be pasteurized in two ways.
- In the older method the milk is held at 63°C for 30 minutes. Large quantities of milk are now usually subjected to **flash pasteurization or high-temperature short-term (HTST)** pasteurization, which consists of quick heating to about 72°C for 15 seconds, then rapid cooling.
- The dairy industry also sometimes uses **ultrahigh-temperature (UHT) sterilization.** Milk and milk products are heated at 140 to 150°C for 1 to 3 seconds. UHT-processed milk does not require refrigeration and can be stored at room temperature for about 2 months without flavor changes. The small coffee creamer portions provided by restaurants often are prepared using UHT sterilization.

- Many objects are best sterilized in the absence of water by **dry heat sterilization.**
- The items to be sterilized are placed in an oven at 160 to 170°C for 2 to 3 hours.
- Microbial death apparently results from denaturation of proteins.
- Although dry air heat is less effective than moist heat— *Clostridium botulinum* spores are killed in 5 minutes at 121°C by moist heat but only after 2 hours at 160°C with dry heat—it has some definite advantages.
- Dry heat does not corrode glassware and metal instruments as moist heat does, and it can be used to sterilize powders, oils, and similar items.
- Most laboratories sterilize glass petri dishes and pipettes with dry heat.
- Despite these advantages, dry heat sterilization is slow and not suitable for heat sensitive materials like many plastic and rubber items.

Low Temperatures

- Often the most convenient control technique is to inhibit their growth and reproduction by the use of either freezing or refrigeration.
- This approach is particularly important in food microbiology. Freezing items at 20°C or lower stops microbial growth because of the low temperature and the absence of liquid water.
- Freezing is a very good method for long-term storage of microbial samples when carried out properly, and many laboratories have a low-temperature freezer for culture storage at 30 or 70°C. Because frozen food can contain many microorganisms, it should be prepared and consumed promptly after thawing in order to avoid spoilage and pathogen growth.
- Refrigeration greatly slows microbial growth and reproduction, but does not halt it completely. Fortunately most pathogens are mesophilic and do not grow well at temperatures around 4°C.
- Thus refrigeration is a good technique only for shorter-term storage of food and other items.

Filtration

- Filtration is an excellent way to reduce the microbial population in solutions of heat-sensitive material, and sometimes it can be used to sterilize solutions. Rather than directly destroying contaminating microorganisms, the filter simply removes them.
- **Membrane filters are now used for many purposes.**
- These circular filters are porous membranes, a little over 0.1 mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate or polyvinylidene fluoride.
- Although a wide variety of pore sizes are available, membranes with pores about 0.2 m in diameter are used to remove most vegetative cells, but not viruses, from solutions ranging in volume from 1 ml to many liters.
- Membrane filters remove microorganisms by screening them out much as a sieve separates large sand particles from small ones.
- These filters are used to sterilize pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics, and other heat-sensitive solutions.
- Air also can be sterilized by filtration. Two common examples are surgical masks and cotton plugs on culture vessels that let air in but keep microorganisms out.

- **Laminar flow biological safety cabinets** employing **high-efficiency particulate air (HEPA) filters,** which remove 99.97% of 0.3 m particles, are one of the most important air filtration systems.
- Laminar flow biological safety cabinets force air through HEPA filters, then project a vertical curtain of sterile air across the cabinet opening. This protects a worker from microorganisms being handled within the cabinet and prevents contamination of the room.
- A person uses these cabinets when working with dangerous agents such as *Mycobacterium tuberculosis,* tumor viruses, and recombinant DNA.
- They are also employed in research labs and industries, such as the pharmaceutical industry, when a sterile working surface is needed for conducting assays, preparing media, examining tissue cultures.

Radiation

- **Ultraviolet (UV) radiation** around 260 nm is quite lethal but does not penetrate glass, dirt films, water, and other substances very effectively. Because of this disadvantage, UV radiation is used as a sterilizing agent only in a few specific situations.

- UV lamps are sometimes placed on the ceilings of rooms or in biological safety cabinets to sterilize the air and any exposed surfaces.

- Because UV radiation burns the skin and damages eyes, people working in such areas must be certain the UV lamps are off when the areas are in use.

- Commercial UV units are available for water treatment. Pathogens and other microorganisms are destroyed when a thin layer of water is passed under the lamps.

The Use of Chemical Agents in Control

- Although objects are sometimes disinfected with physical agents, chemicals are more often employed in disinfection and antisepsis.

- Many factors influence the effectiveness of chemical disinfectants 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. Dirty surfaces must be cleaned before a disinfectant or antiseptic is applied.

- Many different chemicals are available for use as disinfectants, and each has its own advantages and disadvantages.

- In selecting an agent, it is important to keep in mind the characteristics of a desirable disinfectant.

- Ideally the disinfectant must be effective against a wide variety of infectious agents (gram-positive and gram-negative bacteria, acid-fast bacteria, bacterial endospores, fungi, and viruses) at high dilutions.

- Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials.

- In practice, this balance between effectiveness and low toxicity for animals is hard to achieve.

- Some chemicals are used despite their low effectiveness because they are relatively nontoxic.
- The disinfectant should be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms. If possible the disinfectant should be relatively inexpensive.
- One potentially serious problem is the overuse of triclosan and other germicides.
- This antibacterial agent is now found in products such as deodorants, mouthwashes, soaps, cutting boards, and baby toys. Triclosan seems to be everywhere. Thus leading to the emergence of triclosan-resistant bacteria, E.g. *Pseudomonas aeruginosa actively pumps the antiseptic out the cell.*
- Bacteria seem to be responding to antiseptic overuse in the same way as they reacted to antibiotic overuse.
- Thus overuse of antiseptics can have unintended harmful consequences.

Phenolics

- Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during operations.
- Today phenol and phenolics (phenol derivatives) such as cresols, xylenols, and orthophenylphenol are used as disinfectants in laboratories and hospitals.
- The commercial disinfectant Lysol is made of a mixture of phenolics.
- Phenolics act by denaturing proteins and disrupting cell membranes. They have some real advantages as disinfectants: phenolics are tuberculocidal, and remain active on surfaces long after application. However, they do have a disagreeable odor and can cause skin irritation.
- **Hexachlorophene** has been one of the most popular antiseptics because it persists on the skin once applied and reduces skin bacteria for long periods.
- However, it can cause brain damage and is now used in hospital nurseries only in response to a staphylococcal outbreak.

Alcohols

- Alcohols are among the most widely used disinfectants and antiseptics.
- They are bactericidal and fungicidal but not sporicidal; some lipid-containing viruses are also destroyed.
- The two most popular alcohol germicides are ethanol and isopropanol, usually used in about 70 to 80% concentration.
- They act by denaturing proteins and possibly by dissolving membrane lipids.
- A 10 to 15 minute soaking is sufficient to disinfect thermometers and small instruments.

Halogens

- A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VII of the periodic table.

- They exist as diatomic molecules in the free state and form salt like compounds with sodium and most other metals.

- The halogens iodine and chlorine are important antimicrobial agents. Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores.

- Iodine often has been applied as tincture of iodine, 2% or more iodine in a water-ethanol solution of potassium iodide. Although it is an effective antiseptic, the skin may be damaged, a stain is left, and iodine allergies can result.
- More recently iodine has been complexed with solubilizing agent or surfactants to form an **iodophor.**
- **Iodophors** are water soluble, stable, and non staining agents, release iodine slowly to minimize skin burns and irritation. They are used in hospitals for preoperative skin degerming and in hospitals and laboratories for disinfecting.

- **Chlorine** is the usual disinfectant for municipal water supplies and swimming pools and is also employed in the dairy and food industries.
- It may be applied as chlorine gas, sodium hypochlorite, or calcium hypochlorite, all of which yield hypochlorous acid (HClO) and then atomic oxygen.
- Since organic material interferes with chlorine action by reacting with chlorine and its products, an excess of chlorine is added to ensure microbial destruction.
- One potential problem is that chlorine reacts with organic compounds to form carcinogenic trihalomethanes, which must be monitored in drinking water.
- Chlorine is also an excellent disinfectant for individual use because it is effective, inexpensive, and easy to employ.
- Small quantities of drinking water can be disinfected with halazone tablets. Halazone (parasulfone dichloraminobenzoic acid) slowly releases chloride when added to water and disinfects it in about half an hour.
- Chlorine solutions make very effective laboratory and household disinfectants.

Heavy Metals

- For many years the ions of heavy metals such as mercury, silver, arsenic, zinc, and copper were used as germicides.
- More recently these have been superseded by other less toxic and more effective germicides (many heavy metals are more bacteriostatic than bactericidal).
- There are a few exceptions. A 1% solution of silver nitrate is often added to the eyes of infants to prevent ophthalmic gonorrhea (in many hospitals, erythromycin is used instead of silver nitrate because it is effective against Chlamydia as well as Neisseria).
- Silver sulfadiazine is used on burns.
- Copper sulfate is an effective algicide in lakes and swimming pools.
- Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them.
- They may also precipitate cell proteins.

Aldehydes

- Both of the commonly used aldehydes, formaldehyde and glutaraldehyde, are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by crosslinking and alkylating molecules.
- They are sporicidal and can be used as chemical sterilants.
- Formaldehyde is usually dissolved in water or alcohol before use.
- A 2% buffered solution of glutaraldehyde is an effective disinfectant. It is less irritating than formaldehyde and is used to disinfect hospital and laboratory equipment.
- Glutaraldehyde usually disinfects objects within about 10 minutes but may require as long as 12 hours to destroy all spores.

Sterilizing Gases

- Many heat-sensitive items such as disposable plastic petri dishes and syringes, heart-lung machine components are now sterilized with ethylene oxide gas.
- Ethylene oxide (EtO) is both microbicidal and sporicidal and kills by combining with cell proteins.
- It is a particularly effective sterilizing agent because it rapidly penetrates packing materials, even plastic wraps.
- Sterilization is carried out in a special ethylene oxide sterilizer, very much resembling an autoclave in appearance, that controls the EtO concentration, temperature, and humidity.
- Because pure EtO is explosive, it is usually supplied in a 10 to 20% concentration mixed with either CO_2 or dichlorodifluoromethane.
- A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/liter.

- Extensive aeration of the sterilized materials is necessary to remove residual EtO because it is so toxic.

- **Betapropiolactone (BPL)** is occasionally employed as a sterilizing gas.

- In the liquid form it has been used to sterilize vaccines and sera.

- BPL decomposes to an inactive form after several hours and is therefore not as difficult to eliminate as EtO.

- It also destroys microorganisms more readily than ethylene oxide but does not penetrate materials well and may be carcinogenic.

- For these reasons, BPL has not been used as extensively as EtO.