Chapter 22 The Control of Microorganisms by Physical Agents

OUTLINE

The term **control** as used here refers to the reduction in numbers and/or activity of the total microbial flora. The principal reasons for controlling microorganisms are: to prevent transmission of disease and infection, to prevent contamination by or growth of undesirable microorganisms, and to prevent deterioration and spoilage of materials by microorganisms.

Microorganisms can be removed, inhibited, or killed by various **physical agents**, **physical processes**, or **chemical agents**. A variety of techniques and agents are available; they act in many different ways, and each has its own limits of application.

In the first part of this chapter we will consider the fundamentals of control which are applicable when either physical or chemical methods are used. In the second part, we will describe the physical agents and physical methods for reducing or eliminating the viable microbial population.

Before considering the physical means or chemical agents by which microorganisms are controlled, it is important to understand some of the characteristics of a microbial population when exposed to a destructive agent. We shall use bacteria as an example.

FUNDAMENTALS OF CONTROL

The term **death**, as used in microbiology, is defined as the irreversible loss of the ability to reproduce. Viable microorganisms are capable of multiplying; dead microorganisms do not multiply (grow). The determination of death requires laboratory techniques that indicate whether growth occurs when the sample is inoculated into a suitable medium. The failure of a microorganism to grow when inoculated into an appropriate medium indicates that the organism is no longer able to reproduce, and the failure to reproduce is the criterion of death. A complicating factor in this definition is that the response of the organism may not be the same in all media. For example, a suspension of *Escherichia coli*

exposed to a heat treatment may yield a greater number of survivors if a plating medium of trypticase soy agar is used rather than a medium containing bile salts such as deoxycholate agar.

e of Death of

When one drops a suspension of bacteria into a bottle of hot acid or an incinerator, the bacteria may all be killed so fast that it is not possible to measure the death rate. However, less drastic treatment may result in the cells being killed over a longer period of time, at a constant *exponential* rate that is essentially the inverse of their exponential growth pattern.

Exponential death can be understood easily in terms of a simple model. Imagine that each cell is a *target* and that a large number of *bullets* (i.e., units of a physical or chemical agent) are being sprayed at them in a random manner, as with a machine gun; that is, no one is aiming the gun directly at a target. Common sense dictates some rules about the way bacteria die under these conditions. To begin, we assume that a single hit kills a bacterium.

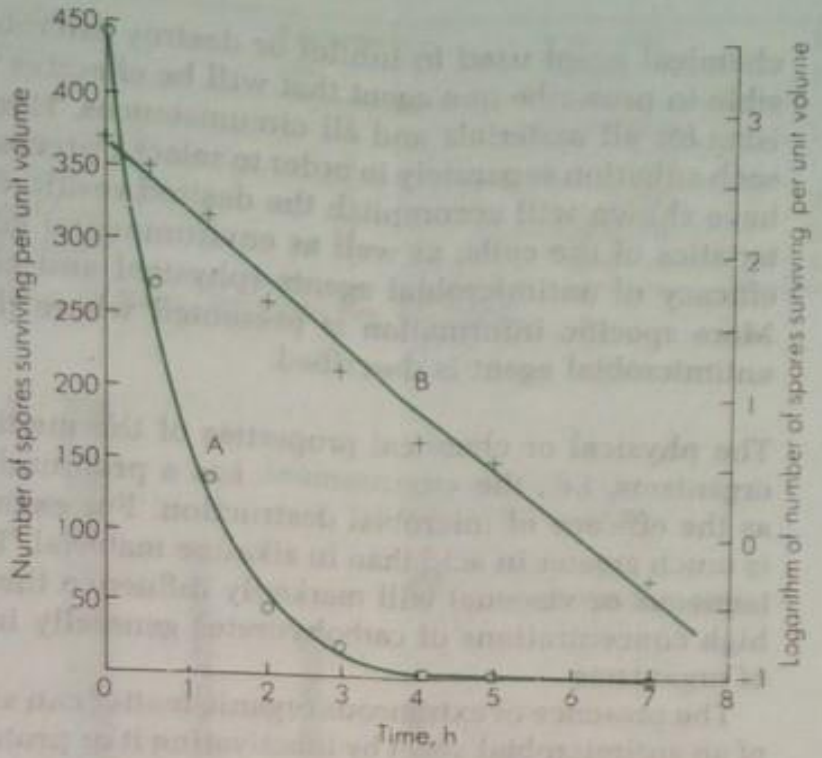
The probability of hitting a target is proportional to the number of targets, i.e., the number of bacteria, present. Intuition tells us that if we shoot randomly at many targets, we have a good chance of hitting one, but as time goes on, the number of targets not yet hit decreases steadily and it becomes harder and harder to hit the remaining ones. (Hitting a target again and again does not count; a bacterium can be killed only once.) Let us take a simple numerical example. Assume that we have an initial population of 1 million targets. We shower them with bullets for 1 min and manage to hit 90 percent, so that there are now 100,000 survivors left. Then we shower them with bullets 1 min more, but since we only have one-tenth as many targets as in the first round, we hit only one-tenth as many. In other words, this time we hit 90,000 of the targets and have 10,000 survivors. We shower these with bullets another minute, and since we have only one-tenth as many as in the last minute, we again hit only one-tenth as many, or 9,000. This pattern repeats itself until there are no targets left, as shown in Table 22-1. But notice that it is just as hard to kill the last nine bacteria as it was to kill the first 900,000. In fact, we can never be sure that we have killed the last one; all that we can do is give the targets enough overkill for there to be a good chance that the last has been hit.

The pattern of death among *Bacillus anthracis* spores exposed to 5% phenol is shown in Fig. 22-1. The number of survivors is plotted against time; however, curve A is an arithmetic plot and curve B is a logarithmic plot. Both curves show that some portion of the population dies during any given unit of time.

2-1. A Theoretical the Order of Death ria When Exposed hal Agent

Time	Survivors	Deaths per Unit Time	Total Deaths
0	1,000,000	0	0
1	100,000	900,000 = 90%	900,000
2	10,000	90,000 = 90%	990,000
3	1,000	9,000 = 90%	999,000
4	100	900 = 90%	999,900
5	10	90 = 90%	999,990
6	1	9 = 90%	999,999

SOURCE: O. Rahn, *Physiology of Bacteria*, McGraw-Hill, New York, 1932.



22-1. The death of *Bacillus anthracis* exposed to 5% phe- Curve A: Number of ors expressed arith- ally per unit volume d against time. Curve garithm of number of ving bacteria plotted st time.

but what the logarithmic plot also reveals is that *the death rate is constant*. The points fall on a straight line, and the slope of the curve is the measure of the death rate.

Results such as those shown in Fig. 22-1 are obtainable only when all conditions are kept strictly uniform, including the age and the physiological condition of all the microorganisms in the population. If the cells in the microbial population vary in age or physiological stage of growth, they will exhibit differences in susceptibility to the agent. As a consequence the logarithmic plot of survivors will not fall in a straight line. Both the slope of the death curve and its form are affected by the species of microorganism and the homogeneity of the cells in the population.

The probability of hitting a target is also *proportional to the number of bullets shot*, i.e., concentration of the chemical or intensity of the physical agent. Intuition again tells us that the more bullets we shoot in a given time, the faster the targets will be hit. If the targets are bacteria and the bullets are x-rays or ultraviolet light, it stands to reason that the cells will be killed faster as the *intensity* of the radiation increases. If the bullets are molecules of some chemical agent, the cells will be killed more rapidly as the *concentration* of the agent increases (up to a certain limit, of course).

The longer we shoot, the more targets we hit, but the more targets we have, the longer it takes to hit them all. This is an obvious restatement of the exponential death pattern. It simply means that it takes time to kill the population, and if we have many cells, we must treat them for a longer time to be reasonably sure that all of the bacteria are dead.

ditions Influencing microbial Action

Microorganisms are not simple physical targets. Many biological characteristics influence the rate at which microorganisms are killed or inactivated by various agents. Many factors must be considered in the application of any physical or

chemical agent used to inhibit or destroy microbial populations. It is not possible to prescribe one agent that will be effective for the control of microorganisms for all materials and all circumstances. Hence it is necessary to evaluate each situation separately in order to select a process that research and experience have shown will accomplish the desired result. Some of the biological characteristics of the cells, as well as environmental conditions which influence the efficacy of antimicrobial agents (physical and chemical), are outlined below. More specific information is presented where the application of a particular antimicrobial agent is described.

vironment

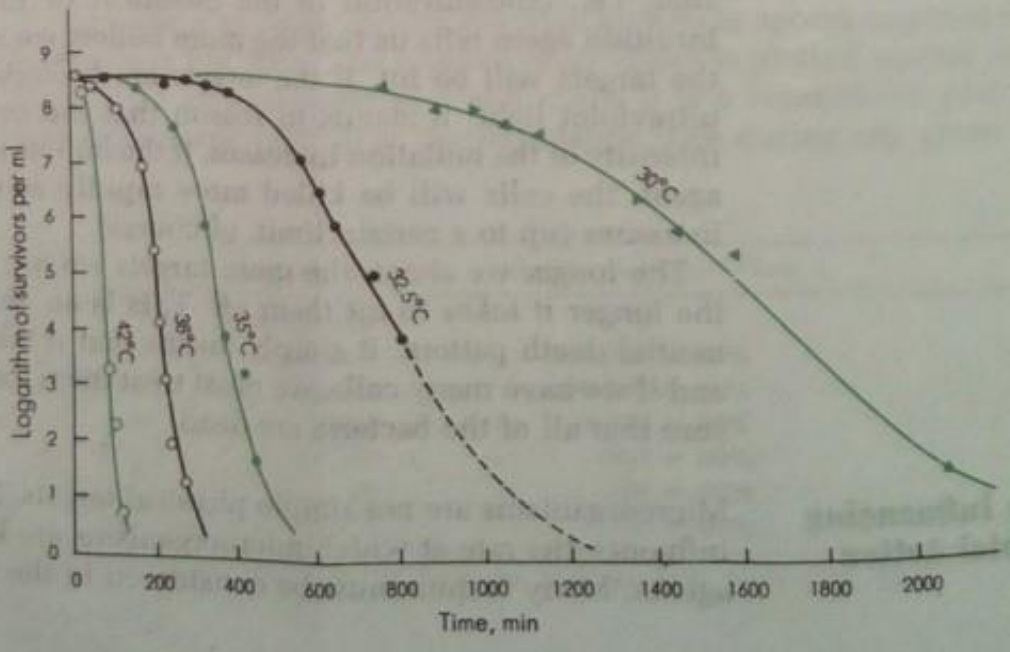
The physical or chemical properties of the medium or substance carrying the organisms, i.e., the environment, has a profound influence on the rate as well as the efficacy of microbial destruction. For example, the effectiveness of heat is much greater in acid than in alkaline material. The consistency of the material (aqueous or viscous) will markedly influence the penetration of the agent, and high concentrations of carbohydrates generally increase the thermal resistance of organisms.

The presence of extraneous organic matter can significantly reduce the efficacy of an antimicrobial agent by inactivating it or protecting the microorganism from it.

An increase in temperature, when used with another agent such as a chemical, hastens the destruction of microorganisms. This phenomenon is illustrated in Fig. 22-2.

inds of Microorganisms

Species of microorganisms differ in their susceptibility to physical and chemical agents. In sporeforming species, the growing vegetative cells are much more susceptible than the spore forms; bacterial spores are extremely resistant. In fact, bacterial spores are the most resistant of all living organisms in their capacity to survive under adverse physical and chemical conditions. The relative resistance of bacterial spores in comparison with other microorganisms is shown in Table 22-2.



igure 22-2. Increasing temerature also decreases bacrial survival when the oncentration of the disinectant remains constant. In his experiment *Escherichia oli* was exposed to phenol t a concentration of 4.62 g/ ter at temperatures beween 30 and 42°C. The umber of survivors, expressed logarithmically, is lotted against time. (*From . C. Jordan and S. E. acobs, J Hyg.* **44**:*210, 1945. Courtesy of Cambridge Uniersity Press.*)

Table 22-2. Resistances of Bacterial and Mold Spores and of Viruses, Relative to the Resistance of *Escherichia coli* as Unity

Sterilizing Agent	*Escherichia coli*	Bacterial Spores	Mold Spores	Viruses and Bacteriophages
Phenol	1	100,000,000	1–2	30
Formaldehyde	1	250		2
Dry heat	1	1,000	2–10	±1
Moist heat	1	3,000,000	2–10	1–5
Ultraviolet	1	2–5	5–100	5–10

SOURCE: O. Rahn, *Bacteriol Rev*, 9:1, 1945.

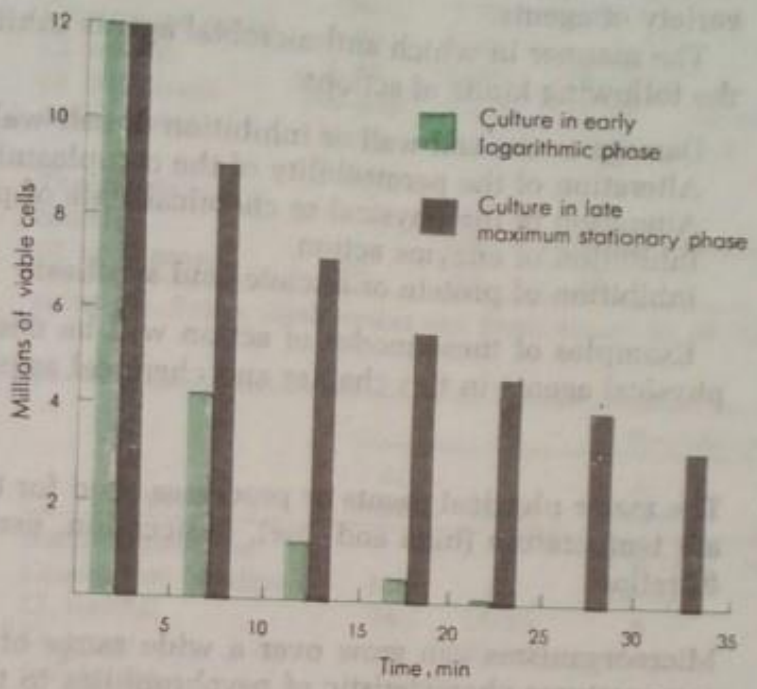


Figure 22-3. Comparative susceptibility of young and old cells to a lethal agent. The young cells are all killed within 25 min, but a considerable part of the more resistant, older cells still survives. (*Courtesy of Martin Frobisher et al., Fundamentals of Microbiology, Saunders, Philadelphia, 1974.*)

Physiological State of Cells

The physiological state of cells may influence susceptibility to an antimicrobial agent. Young, actively metabolizing cells are apt to be more easily destroyed than old, dormant cells in the case of an agent that causes damage through the interference with metabolism; nongrowing cells would not be affected. A comparison of the susceptibility of young and old cells to a lethal agent is shown in Fig. 22-3.

Mode of Action of Antimicrobial Agents

The many processes and substances used as antimicrobial agents manifest their activity in one of several ways. For both academic and practical reasons, it is important to know how microorganisms are inhibited or killed. Knowledge of the mode of action of a particular agent may make it possible to predict the conditions under which it will function most effectively as well as the kinds of microorganisms it will be most effective against. A great deal of research has been performed to determine the specific site of action of various agents. Such investigations are complicated by the fact that when a cell is exposed to a lethal agent, many changes can be observed. A domino effect occurs once the initial inhibitory or lethal process is inflicted. The real problem is to establish the *primary site of damage* responsible for inhibition or death.

In a general way, one may view the possible sites of action of an antimicrobial agent by recalling certain features of the microbial cell. A normal living cell

contains the multitude of enzymes responsible for metabolic processes. A semipermeable membrane (cytoplasmic membrane) maintains the integrity of the cellular contents; the membrane selectively controls the passage of substances between the cell and its external environment and is also the site of some enzyme reactions. The cell wall proper provides a protective covering to the cell in addition to participating in certain physiological processes. Damage at any of these areas may initiate a number of subsequent changes leading to the death of the cell. It is necessary to bear in mind that there are many sites of damage to the cell and that the damage may be caused by one or more of a variety of agents.

The manner in which antimicrobial agents inhibit or kill can be attributed to the following kinds of actions:

Damage to the cell wall or inhibition of cell-wall synthesis
Alteration of the permeability of the cytoplasmic membrane
Alteration of the physical or chemical state of proteins and nucleic acids
Inhibition of enzyme action
Inhibition of protein or nucleic acid synthesis

Examples of these modes of action will be described as we discuss specific physical agents in this chapter and chemical agents in Chaps. 23 and 24.

PHYSICAL AGENTS

The major physical agents or processes used for the control of microorganisms are temperature (high and low), desiccation, osmotic pressure, radiation, and filtration.

High Temperatures

Microorganisms can grow over a wide range of temperatures, from very low temperatures characteristic of psychrophiles to the very high growth temperatures characteristic of thermophiles. Every type has an optimum, minimum, and maximum growth temperature. Temperatures above the maximum generally kill, while those below the minimum usually produce stasis (inhibition of metabolism) and may even be considered preservative. The amount of water present in the environment at any temperature has a significant effect upon microorganisms in terms of their survival.

High temperatures combined with high moisture is one of the most effective methods of killing microorganisms. It is important to distinguish between dry heat and moist heat in any procedure for microbial control. Moist heat kills microorganisms by coagulating their proteins and is much more rapid and effective than dry heat, which destroys microorganisms by oxidizing their chemical constituents. Two examples will illustrate the difference. Spores of *Clostridium botulinum* are killed in 4 to 20 min by moist heat at 120°C, whereas a 2-h exposure to dry heat at the same temperature is required. Spores of *B. anthracis* are destroyed in 2 to 15 min by moist heat at 100°C, but with dry heat 1 to 2 h at 150°C is required to achieve the same result (Tables 22-3 and 22-4).

Vegetative cells are much more sensitive to heat than are spores; the higher level of "water activity" in the vegetative cells accounts for this. Cells of most bacteria are killed in 5 to 10 min at 60 to 70°C (moist heat). Vegetative cells of yeasts and other fungi are usually killed in 5 to 10 min by moist heat at 50 to

Table 22-3. Some Quoted Destruction Times of Bacterial Spores by Moist Heat

Organism	Destruction times, min							
	At 100°C	At 105°C	At 110°C	At 115°C	At 120°C	At 125°C	At 130°C	At 134°C
Bacillus anthracis	2–15	5–10						
B. subtilis	Many hours			40				
A putrefactive anaerobe	780	170	41	15	5.6			
Clostridium tetani	5–90	5–25						
Cl. welchii	5–45	5–27	10–15	4	1			
Cl. botulinum	300–530	40–120	32–90	10–40	4–20			
Soil bacteria	Many hours	420	120	15	6–30	4		1.5–10
Thermophilic bacteria		400	100–300	40–110	11–35	3.9–8.0	3.5	1
Cl. sporogenes	150	45	12					

SOURCE: G. Sykes, *Distinfection and Sterilization*, 2d ed., Lippincott, Philadelphia, 1965.

Table 22-4. Some Quoted Killing Times of Bacterial Spores by Dry Heat

Organism	Destruction times, min						
	At 120°C	At 130°C	At 140°C	At 150°C	At 160°C	At 170°C	At 180°C
Bacillus anthracis			Up to 180	60–120	9–90		3
Clostridium botulinum	120	60	15–60	25	20–25	10–15	5–10
Cl. welchii	50	15–35	5				
Cl. tetani		20–40	5–15	30	12	5	1
Soil spores				180	30–90	15–60	15

SOURCE: G. Sykes, *Disinfection and Sterilization*, 2d ed., Lippincott, Philadelphia, 1965.

60°C; their spores are killed in the same time but at temperatures of 70 to 80°C. Bacterial spores are much more resistant to high temperatures as shown in Tables 22-3 and 22-4. The susceptibility of viruses to heat is generally similar to that of mesophilic vegetative bacterial cells.

Thermal Death Time and Decimal Reduction Time

Thermal death time refers to the shortest period of time to kill a suspension of bacteria (or spores) at a prescribed temperature and under specific conditions. Another unit of measurement of the destruction of microorganisms by heat is the **decimal reduction time**. This is the time in minutes to reduce the population by 90 percent, or stated differently, it is the time in minutes for the thermal-death-time curve to pass through one log cycle (see Fig. 22-4). Figure 22-5 illustrates a thermal-death-time curve for spores of bacterial species responsible for a type of canned-food spoilage, e.g., flat sour spoilage.

From the definition of these terms, it is clear that they express a time-temperature relationship to killing. In thermal death time, the temperature is selected as the fixed point and the time varied. Decimal reduction time is a modification of thermal death time which measures a 90 percent rather than 100 percent kill rate. In the experimental determination of these values, it is an absolute requirement that the conditions be rigidly controlled. Attention must

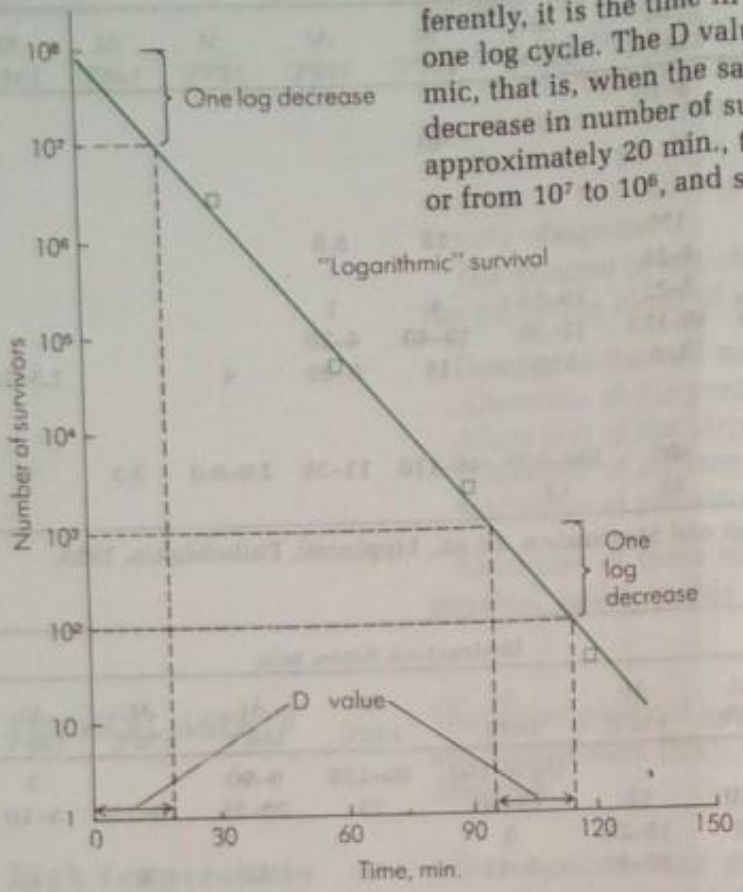


Figure 22-4. Graph illustrating the concept of decimal reduction time (D value), the time in minutes to reduce the microbial population by 90 percent. Stated differently, it is the time in minutes for the thermal-death-time curve to pass through one log cycle. The D value is independent of time when the response is logarithmic, that is, when the same length of time is required to accomplish any given log decrease in number of survivors. For example, the D value in this illustration is approximately 20 min., the time required to reduce the survivors from 10^8 to 10^7, or from 10^7 to 10^6, and so on.

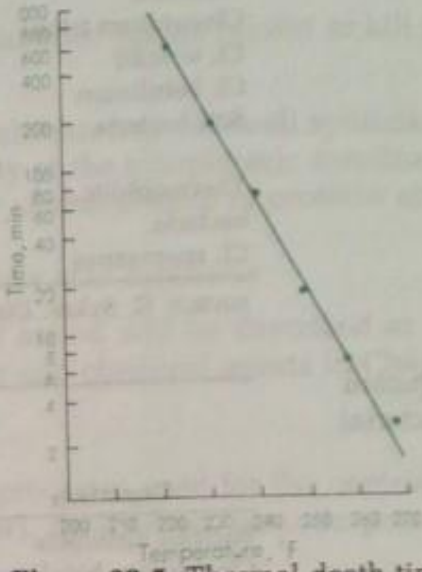


Figure 22-5. Thermal-death-time curve for spores of a type of bacterium encountered in food spoilage. (*Courtesy of W. C. Frazier, Food Microbiology, McGraw-Hill, New York, 1958.*)

be given to the nature of the medium, pH, and the number of organisms, since all these factors have a bearing on the susceptibility of the microorganisms to heat.

Thermal-death-time data and decimal-reduction-time data are extremely important in many applications of microbiology. The canning industry, for example, carries out extensive studies on this subject to establish satisfactory processing temperatures for the preservation of canned foods.

Application of High Temperatures for Destruction of Microorganisms

The killing action of heat is, as we have seen, a time-temperature relationship affected by numerous conditions that must be taken into consideration in selecting the time and temperature required to reduce the microbial population to the desired level. Practical procedures by which heat is employed are conveniently divided into two categories: moist heat and dry heat.

Moist Heat. The application of moist heat for inhibiting or destroying microorganisms is discussed by the method used to obtain the desired result.

Steam Under Pressure. Heat in the form of saturated steam under pressure is the most practical and dependable agent for sterilization. Steam under pressure provides temperatures above those obtainable by boiling, as shown in Table 22-5. In addition, it has the advantages of rapid heating, penetration, and moisture in abundance, which facilitates the coagulation of proteins.

The laboratory apparatus designed to use steam under regulated pressure is

ble 22-5. Temperature of eam under Pressure

eam Pressure, lb/in^2	Temperature, °C
0	100.0
5	109.0
10	115.0
15	121.5
20	126.5

URCE: J. J. Perkins, *Principles nd Methods of Sterilization*, harles C. Thomas, Springfield, L, 1956.

called an autoclave (see Fig. 22-6). It is essentially a double-jacketed steam chamber equipped with devices which permit the chamber to be filled with saturated steam and maintained at a designated temperature and pressure for any period of time. In the operation of an autoclave it is absolutely essential that the air in the chamber be completely replaced by saturated steam. If air is present, it will reduce the temperature obtained within the chamber substantially below that which would be realized if pure saturated steam were under the same pressure. It is not the pressure that kills the organisms but the *temperature* of the steam.

The autoclave is an essential unit of equipment in every microbiology laboratory. Many media, solutions, discarded cultures, and contaminated materials are routinely sterilized with this apparatus. Generally, but not always, the autoclave is operated at a pressure of approximately 15 lb/in^2 (at 121°C). The time of operation to achieve sterility depends on the nature of the material being sterilized, the type of the container, and the volume. For example, 1000 test tubes containing 10 ml each of a liquid medium can be sterilized in 10 to 15 min at 121°C; 10 liters of the same medium contained in a single container would require 1 h or more at the same temperature to ensure sterilization.

Fractional Sterilization. Some microbiological media, solutions of chemicals, and biological materials cannot be heated above 100°C without being damaged. If, however, they can withstand the temperature of free-flowing steam (100°C), it is possible to sterilize them by fractional sterilization (tyndallization). This method involves heating the material at 100°C on three successive days with incubation periods in between. Resistant spores germinate during the incubation

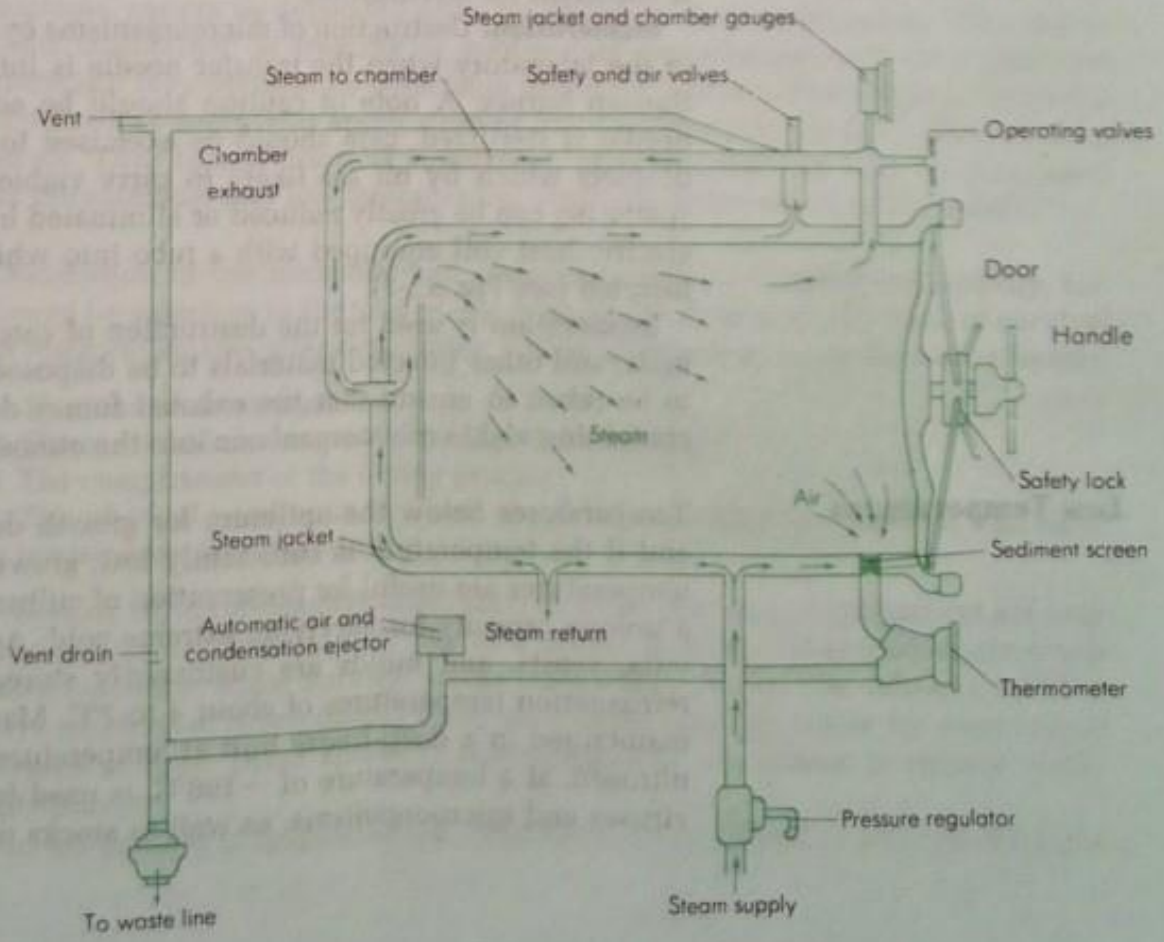


Figure 22-6. Pressure steam sterilizer (autoclave), cross-sectional view illustrating operational parts and path of steam flow. *(Courtesy of Wilmont Castle Company.)*

periods; on subsequent exposure to heat, the vegetative cells will be destroyed. If spores are present and do not germinate during the incubation periods, the material will not be sterilized. An apparatus known as the Steam Arnold is used for this technique; however, it is also possible to operate an autoclave with free-flowing steam for this purpose.

Boiling Water. Contaminated materials or objects exposed to boiling water cannot be sterilized with certainty. It is true that all vegetative cells will be destroyed within minutes by exposure to boiling water, but some bacterial spores can withstand this condition for many hours. The practice of exposing instruments for short periods of time in boiling water is more likely to bring about disinfection (destruction of vegetative cells of disease-producing microorganisms) rather than sterilization. Boiling water cannot be (and is not) used in the laboratory as a method of sterilization.

Pasteurization. Milk, cream, and certain alcoholic beverages (beer and wine) are subjected to a controlled heat treatment (called pasteurization) which kills microorganisms of certain types but does not destroy all organisms. Pasteurized milk is not sterile milk. The pasteurization of milk is discussed in Chap. 28.

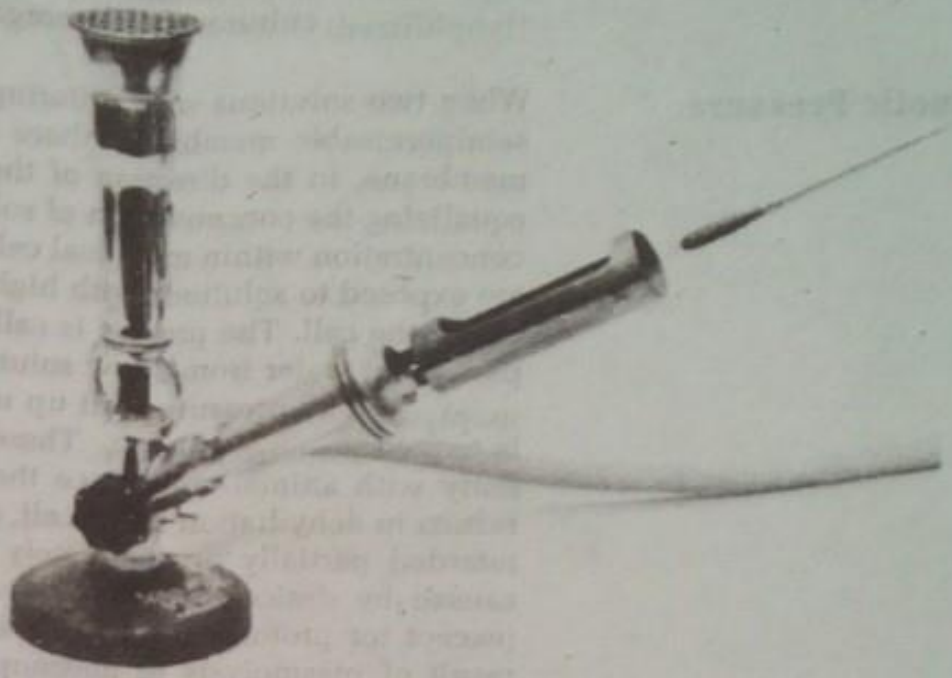
Dry-Heat: *Hot-Air Sterilization.* Dry-heat, or hot-air, sterilization is recommended where it is either undesirable or unlikely that steam under pressure will make direct and complete contact with the materials to be sterilized. This is true of certain items of laboratory glassware, such as Petri dishes and pipettes, as well as oils, powders, and similar substances. The apparatus employed for this type of sterilization may be a special electric or gas oven or even the kitchen stove oven. For laboratory glassware, a 2-h exposure to a temperature of 160°C is sufficient for sterilization.

Incineration. Destruction of microorganisms by burning is practiced routinely in the laboratory when the transfer needle is introduced into the flame of the Bunsen burner. A note of caution should be added here. When the transfer needle is sterilized, care should be exercised to prevent spattering, since the droplets which fly off are likely to carry viable organisms. The danger from spattering can be greatly reduced or eliminated by using a Bunsen burner or an electric heat coil equipped with a tube into which the transfer needle can be inserted (see Fig. 22-7).

Incineration is used for the destruction of carcasses, infected laboratory animals, and other infected materials to be disposed of. Special precautions need to be taken to ensure that the exhaust fumes do not carry particulate matter containing viable microorganisms into the atmosphere.

ratures

Temperatures below the optimum for growth depress the rate of metabolism, and if the temperature is sufficiently low, growth and metabolism cease. Low temperatures are useful for preservation of cultures, since microorganisms have a unique capacity for surviving extreme cold. Agar-slant cultures of some bacteria, yeasts, and molds are customarily stored for long periods of time at refrigeration temperatures of about 4 to 7°C. Many bacteria and viruses can be maintained in a deep-freeze unit at temperatures from −20 to −70°C. Liquid nitrogen, at a temperature of −196°C, is used for preserving cultures of many viruses and microorganisms, as well as stocks of mammalian tissue cells used



igure 22-7. When a transfer eedle is placed in a flame, pattering may occur with sultant spread of living or- anisms. To prevent this, ne can use a Bunsen urner which is modified so at the transfer needle is xposed to a flame within a ibular space, as shown ere.

in animal virology and many other types of research. In all these procedures, the initial freezing kills a fraction of the population, but the survivors may remain viable for long periods. (See Chap. 8.)

From these facts it is immediately apparent that low temperatures, however extreme, cannot be depended upon for disinfection or sterilization. Microorganisms maintained at freezing or subfreezing temperatures may be considered dormant; they perform no detectable metabolic activity. This static condition is the basis of successful application of low temperatures for the preservation of foods. Thus from a practical standpoint, high temperatures may be considered as microbicidal and low temperatures (freezing or lower) as microbistatic.

esiccation

Desiccation of the microbial cell causes a cessation of metabolic activity, followed by a decline in the total viable population. In general, the time of survival of microorganisms after desiccation varies, depending on the following factors:

1 The kind of microorganism
2 The material in or on which the organisms are dried
3 The completeness of the drying process
4 The physical conditions to which the dried organisms are exposed, e.g., light, temperature, and humidity

Species of Gram-negative cocci such as gonococci and meningococci are very sensitive to desiccation; they die in a matter of hours. Streptococci are much more resistant; some survive weeks after being dried. The tubercle bacillus (*Mycobacterium tuberculosis*) dried in sputum remains viable for even longer periods of time. Dried spores of microorganisms are known to remain viable indefinitely.

In the process of lyophilization, organisms are subjected to extreme dehydra-

tion in the frozen state and then sealed in a vacuum. In this condition, desiccated (lyophilized) cultures of microorganisms remain viable for many years.

Osmotic Pressure

When two solutions with differing concentrations of solute are separated by a semipermeable membrane, there will occur a passage of water, through the membrane, in the direction of the higher concentration. The trend is toward equalizing the concentration of solute on both sides of a membrane. The solute concentration within microbial cells is approximately 0.95 percent. Thus if cells are exposed to solutions with higher solute concentration, water will be drawn out of the cell. The process is called **plasmolysis**. The reverse process, that is, passage of water from a low solute concentration into the cell, is termed **plasmoptysis**. The pressure built up within the cell as a result of this water intake is termed **osmotic pressure**. These phenomena can be observed more conveniently with animal cells since they do not have rigid cell walls. Plasmolysis results in dehydration of the cell, and as a consequence metabolic processes are retarded partially or completely. The antimicrobial effect is similar to that caused by desiccation. Because of the great rigidity of microbial cell walls (except for protozoa), the cell-wall structure does not exhibit distortions as a result of plasmolysis or plasmoptysis. However, changes in the cytoplasmic membrane, and particularly shrinkage of the protoplast from the cell wall, can be observed during plasmolysis.

Radiation

Energy transmitted through space in a variety of forms is generally called **radiation**. For our purposes, the most significant type of radiation is probably **electromagnetic radiation**, of which light and x-rays are examples. Electromagnetic radiation has the dual properties of a *continuous* wave phenomenon and a *discontinuous* particle phenomenon; the particles are packets, or **quanta**, of energy, sometimes called **photons**, which vibrate at different frequencies. Radiation of a given frequency can also be described by its wavelength, λ; it is measured in angstroms, where 10,000 Å = 1 μm, and the energy of the radiation in electron volts (ev) is given by $12{,}350/\lambda$. The various parts of the electromagnetic spectrum, distinguished by their wavelengths, are shown in Fig. 22-8.

Electromagnetic radiation can interact with matter in one of two general ways

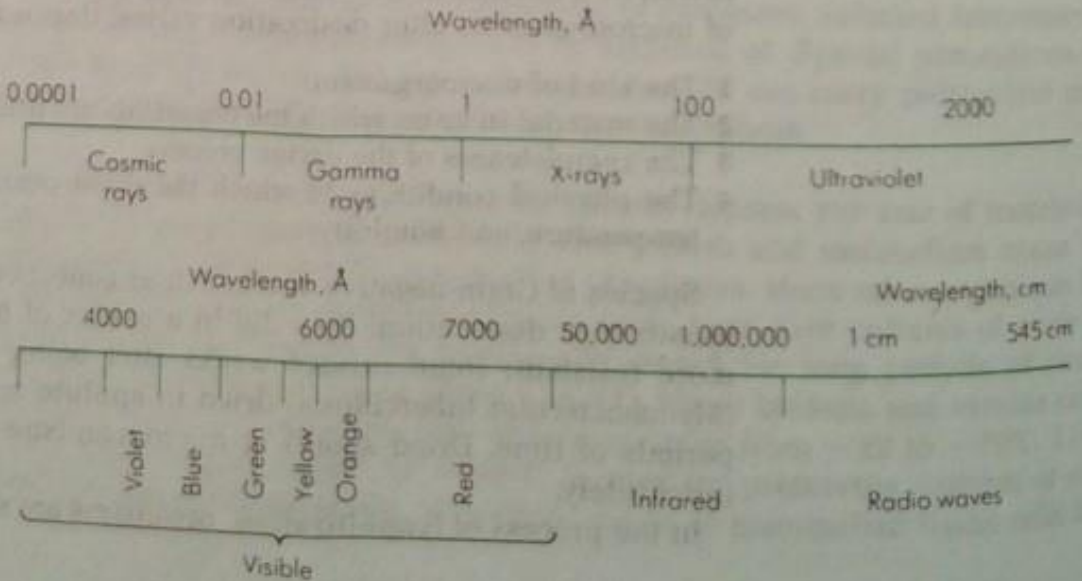


Figure 22-8. Spectrum of radiant energy. (1 Å = 0.1 nm.)

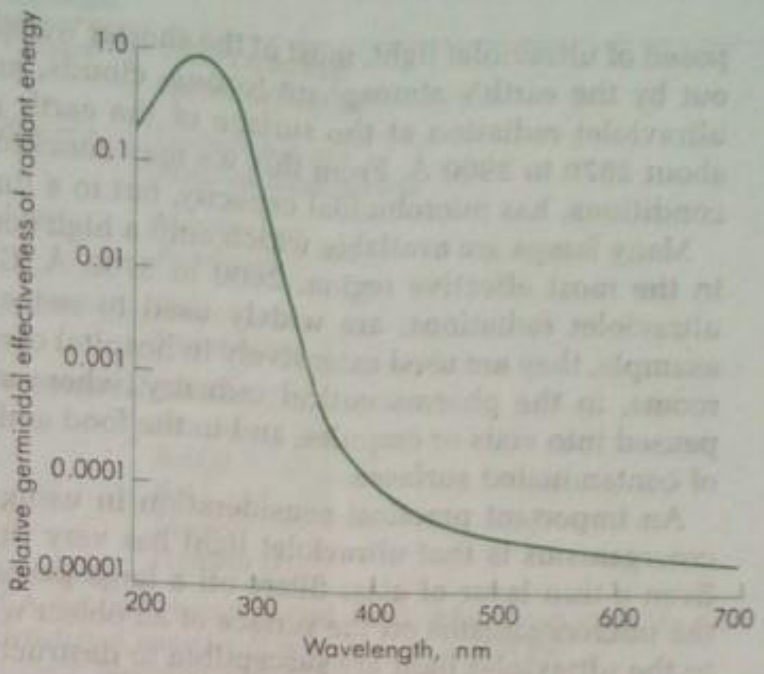


Figure 22-9. Relative germicidal effectiveness of radiant energy between 2000 and 7000 Å. *(Courtesy of General Electric Company, Lamp Division, Publication LD-11.)*

Gamma rays and x-rays, which have energies of more than about 10 eV, are called ionizing radiations because they have enough energy to knock electrons away from molecules and ionize them. When such radiations pass through cells, they create free hydrogen radicals, hydroxyi radicals, and some peroxides, which in turn can cause different kinds of intracellular damage. Moreover, since this damage is produced in a variety of materials, ionizing radiations are rather nonspecific in their effects. Less energetic radiation, particularly ultraviolet light, does not ionize; it is absorbed quite specifically by different compounds because it excites electrons and raises them to higher energy levels, thus creating different chemical species that can engage in a variety of chemical reactions not possible for unexcited molecules.

In addition to electromagnetic radiation, organisms may be subjected to acoustic radiation (sound waves) and to subatomic particles, such as those released in radioactive decay. The atomic era has alerted us to the damaging potential of radiation. Consequently, a tremendous expenditure of research effort is being directed toward determining the minimum dosage which affects cells, how radiations damage cells, and how the damage can be prevented. Microorganisms have been used for the major part of this research for the same reasons they are used in so many other areas of basic biological research; they are easy to grow and lend themselves to rapid, efficient experimentation.

Besides the fundamental research in radiation microbiology, there have been many developments in the application of ionizing radiation to sterilize biological materials. This method is called cold sterilization because ionizing radiations produce relatively little heat in the material being irradiated. Thus it is possible to sterilize heat-sensitive substances, and such techniques are being developed in the food and pharmaceutical industries.

Ultraviolet Light

The ultraviolet portion of the spectrum (Fig. 22-8) includes all radiations from 150 to 3900 Å. Wavelengths around 2650 Å have the highest bactericidal efficiency (see Fig. 22-9). Although the radiant energy of sunlight is partly com-

posed of ultraviolet light, most of the shorter wavelengths of this type are filtered out by the earth's atmosphere (ozone, clouds, and smoke). Consequently, the ultraviolet radiation at the surface of the earth is restricted to the span from about 2670 to 3900 Å. From this we may conclude that sunlight, under certain conditions, has microbicidal capacity, but to a limited degree.

Many lamps are available which emit a high concentration of ultraviolet light in the most effective region, 2600 to 2700 Å. Germicidal lamps, which emit ultraviolet radiations, are widely used to reduce microbial populations. For example, they are used extensively in hospital operating rooms, in aseptic filling rooms, in the pharmaceutical industry, where sterile products are being dispensed into vials or ampules, and in the food and dairy industries for treatment of contaminated surfaces.

An important practical consideration in using this means of destroying microorganisms is that ultraviolet light has very little ability to penetrate matter. Even a thin layer of glass filters off a large percentage of the light. Thus, only the microorganisms on the surface of an object where they are exposed directly to the ultraviolet light are susceptible to destruction.

Mode of Action. Ultraviolet light is absorbed by many cellular materials but most significantly by the nucleic acids, where it does the most damage. The absorption and subsequent reactions are predominantly in the pyrimidines of the nucleic acid. One important alteration is the formation of a *pyrimidine dimer* in which two adjacent pyrimidines become bonded. Unless dimers are removed by specific intracellular enzymes, DNA replication can be inhibited and mutations can result. (See Chap. 12.)

-Rays (Roentgen Rays)

X-rays are lethal to microorganisms and higher forms of life (see Table 22-6). Unlike ultraviolet radiations, they have considerable energy and penetration ability. However, they are impractical for purposes of controlling microbial populations because (1) they are very expensive to produce in quantity and (2) they are difficult to utilize efficiently, since radiations are given off in all directions from their point of origin. However, x-rays have been widely employed experimentally to produce microbial mutants, as mentioned in Chap. 12.

amma Rays

Gamma radiations are high-energy radiations emitted from certain radioactive isotopes such as ^{60}Co. As a result of the major research programs with atomic energy, large quantities of radioisotopes have become available as by-products of atomic fission. These isotopes are potential sources of gamma radiations. Gamma rays are similar to x-rays but are of shorter wavelength and higher energy. They are capable of great penetration into matter, and they are lethal to all life, including microorganisms.

Because of their great penetrating power and their microbicidal effect, gamma rays are attractive for use in commercial sterilization of materials of considerable thickness or volume, e.g., packaged foods and medical devices. However, certain technical problems must be resolved for practical applications, e.g., development of radiation sources for large-scale use and the design of equipment to eliminate any possible hazards to the operators.

Results of quantitative studies on the effect of ionizing radiations on the cells

Table 22-6. Median Lethal Dose of X-Rays for Various Species of Organisms

Organism		Median Lethal Dose, rd*
Viruses:	Tobacco mosaic	200,000
	Rabbit papilloma	100,000
Bacteria:	*Escherichia coli*	5,000
	Bacillus mesentericus	130,000
Algae:	*Mesotenium*	8,500
	Pandorina	4,000
Protozoa:	*Colpidium*	330,000
	Paramecium	300,000
Vertebrates:	Goldfish	750
	Mouse	450
	Rabbit	800
	Rat	600
	Monkey	450
	Humans (?)	400

* A rad (radiation absorbed dose), abbreviated rd, is the dose which delivers 100 ergs/g of irradiated material; it is equal to 6×10^{13} eV.

SOURCE: Modified from E. Paterson, in R. Paterson (ed.), *The Treatment of Malignant Disease by Radium and X Rays*, E. Arnold, London, 1948; *McGraw-Hill Encyclopedia of Science and Technology*, vol. 11, p. 244, McGraw-Hill, New York, 1971.

have resulted in the establishment of the "target" theory of action. This implies that the radiant-energy particle makes a "direct hit" on some essential substance such as DNA within the bacterial cell, causing ionization which results in the death of the cell.

Cathode Rays (Electron-Beam Radiation)

When a high-voltage potential is established between a cathode and an anode in an evacuated tube, the cathode emits beams of electrons, called cathode rays or electron beams. Special types of equipment have been designed which produce electrons of very high intensities (millions of volts), and these electrons are accelerated to extremely high velocities. These intense beams of accelerated electrons are microbicidal as well as having other effects on biological and nonbiological materials.

The electron accelerator, a type of equipment which produces the high-voltage electron beam, is used today for the sterilization of surgical supplies, drugs, and other materials. One of the unique features of the process is that the material can be sterilized after it has been packaged (the radiations penetrate the wrappings) and at room temperature. Electron-beam radiation has limited power of penetration; but within its limits of penetration, sterilization is accomplished on very brief exposure.

The susceptibility of microorganisms to doses of different radiations is shown in Table 22-7.

Surface Tension and Interfacial Tension

The interface, or boundary, between a liquid and a gas is characterized by unbalanced forces of attraction between the molecules in the surface of the liquid and in the interior. A molecule at the surface of the liquid-air interface is pulled strongly toward the interior of the liquid beneath it, whereas

ble 22-7. Lethal Doses of
fferent Radiations

Type of Organism	Lethal Doses, mrd			
	Cathode Rays from van de Graaff Accelerators*	From: Capacitron Pulsed Beam†	Gamma Rays from ^{60}Co	X-rays from 3-MeV Source
Vegetative:				
Nonpathogenic	0.1–0.25			
Pathogenic	0.45–0.55	0.1–0.25	0.15–0.25	0.03–0.5
Bacterial spores	0.5–2.1	0.2–0.4	1.5‡	0.5–2.0
Molds	0.25–1.15	0.35–0.4	0.2–0.3	0.25–1.0
Yeasts	0.5–1.0		0.3	0.25–1.5

* Various authors.
† Huber and colleagues, quoted by Hannan, 1955.
‡ Approximate.
SOURCE: G. Sykes, "Methods and Equipment for Sterilization of Laboratory Apparatus and Media," in J. R. Norris and D. W. Ribbons (eds.), *Methods in Microbiology*, vol. 1, Academic, New York, 1969.

molecules in the interior of the liquid are attracted uniformly in all directions. This behavior of molecular forces at the liquid-air interface imparts a distinctive characteristic to the surface of a liquid, known as **surface tension**. Surface forces also exist between two immiscible liquids and at the interface between a solid and a liquid. Here they are referred to as **interfacial tension**. Changes in surface tension may alter the permeability charcteristics of the cytoplasmic membrane, causing leakage of cellular substances, which results in damage to the cell.

ltration

cteriological Filters

For many years a variety of filters have been available to the microbiologist which can remove microorganisms from liquids or gases. These filters are made of different materials—an asbestos pad in the Seitz filter, diatomaceous earth in the Berkefeld filter, porcelain in the Chamberland-Pasteur filter, and sintered glass disks in other filters.

The mean pore diameter in these bacteriological filters ranges from approximately one to several micrometers; most filters are available in several grades, based on the average size of the pores. However, it should be understood that these filters do not act as mere mechanical sieves; porosity alone is not the only factor preventing the passage of organisms. Other factors, such as the electric charge of the filter, the electric charge carried by the organisms, and the nature of the fluid being filtered, can influence the efficiency of filtration.

In recent years a new type of filter termed the **membrane** or **molecular filter** has been developed whose pores are of a uniform and specific predetermined size. Membrane or molecular filters are composed of biologically inert cellulose esters. They are prepared as circular membranes of about 150-μm thickness and contain millions of microscopic pores of very uniform diameter (see Fig. 22-10). Filters of this type can be produced with known porosities ranging from approximately 0.01 to 10 μm. Membrane filters are used extensively in the laboratory and in industry to sterilize fluid materials. They have been adapted to microbiological procedures for the identification and enumeration of microorganisms from water samples and other materials (see Chap. 26.)

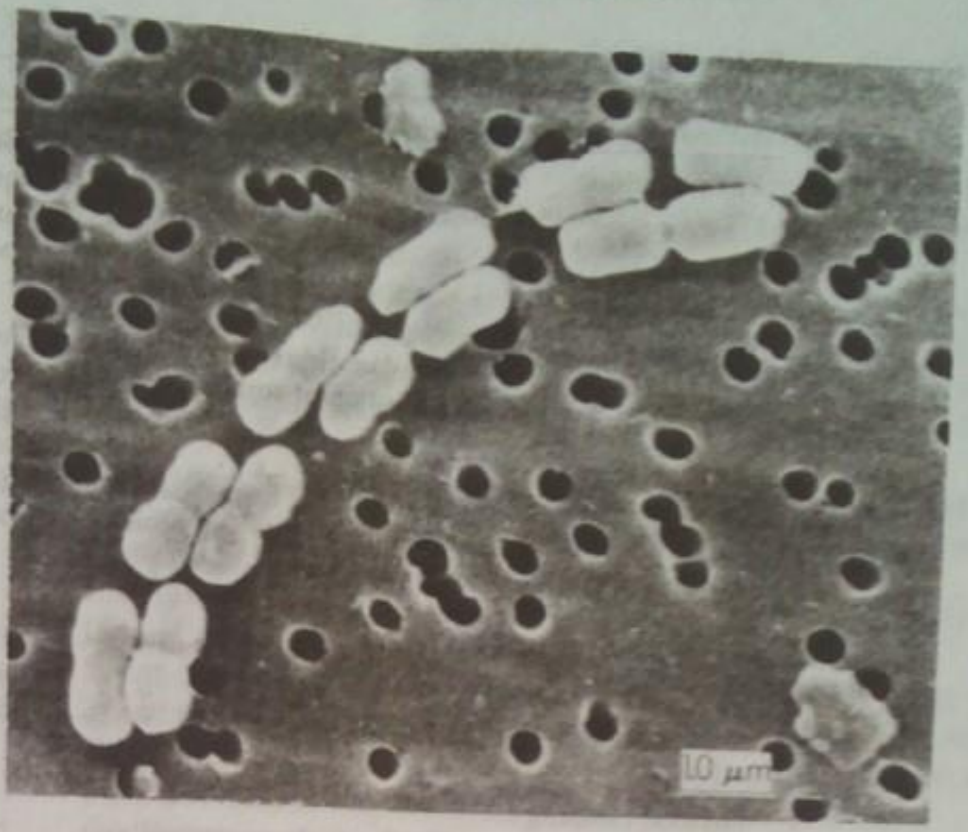


Figure 22-10. Bacteria from a marine water sample are retained by this membrane filter. (*Courtesy of Pall Corporation.*)

It is customary to force the fluid through the filter by applying a negative pressure to the filter flask by use of a vacuum or water pump or to impose a positive pressure above the fluid in the filter chamber, thus forcing it through. Upon completion of filtration, precautions must be taken to prevent contamination of the filtered material when it is transferred to other containers.

The development of high-efficiency particulate air (HEPA) filters has made it possible to deliver clean air to an enclosure such as a cubicle or a room. This type of air filtration together with a system of laminar airflow is now used extensively to produce dust- and bacteria-free air. (See. Fig. 22-11.)

A summary of the application of physical agents for the control of microorganisms is provided in Table 22-8.

Table 22-8. Application of Physical Agents for Controlling Microorganisms

Method	Recommended Uses	Limitations
Moist heat		
Autoclave	Sterilizing instruments, linens, utensils, and treatment trays, media and other liquids	Ineffective against organisms in materials impervious to steam; cannot be used for heat-sensitive articles
Free-flowing steam or boiling water	Destruction of nonsporeforming pathogens; sanitizes bedding, clothing, and dishes	Cannot be guaranteed to produce sterilization on one exposure
Dry heat		
Hot-air oven	Sterilizing materials impermeable to or damaged by moisture, e.g., oils, glass, sharp instruments, metals	Destructive to materials which cannot withstand high temperatures for long periods

Table 22.8. *(continued)*

Method	Recommended Uses	Limitations
Incineration	Disposal of contaminated objects that cannot be reused.	Size of incinerator must be adequate to burn largest load promptly and completely; potential of air pollution
Radiation		
Ultraviolet light	Control of airborne infection; disinfection of surfaces	Must be absorbed to be effective (does not pass through transparent glass or opaque objects); irritating to eyes and skin; low penetration
X-ray, gamma, and cathode radiation	Sterilization of heat-sensitive surgical materials and other medical devices	Expensive and requires special facilities for use
Filtration		
Membrane filters	Sterilization of heat-sensitive biological fluids	Fluid must be relatiely free of suspended particulate matter
Fiberglass filters (HEPA)	Air disinfection	Expensive
Physical cleaning		
Ultrasonics	Effective in decontaminating delicate cleaning instruments	Not effective alone, but as adjunct procedure enhances effectiveness of other methods
Washing	Hands, skin, objects	Sanitizes; reduces microbial flora

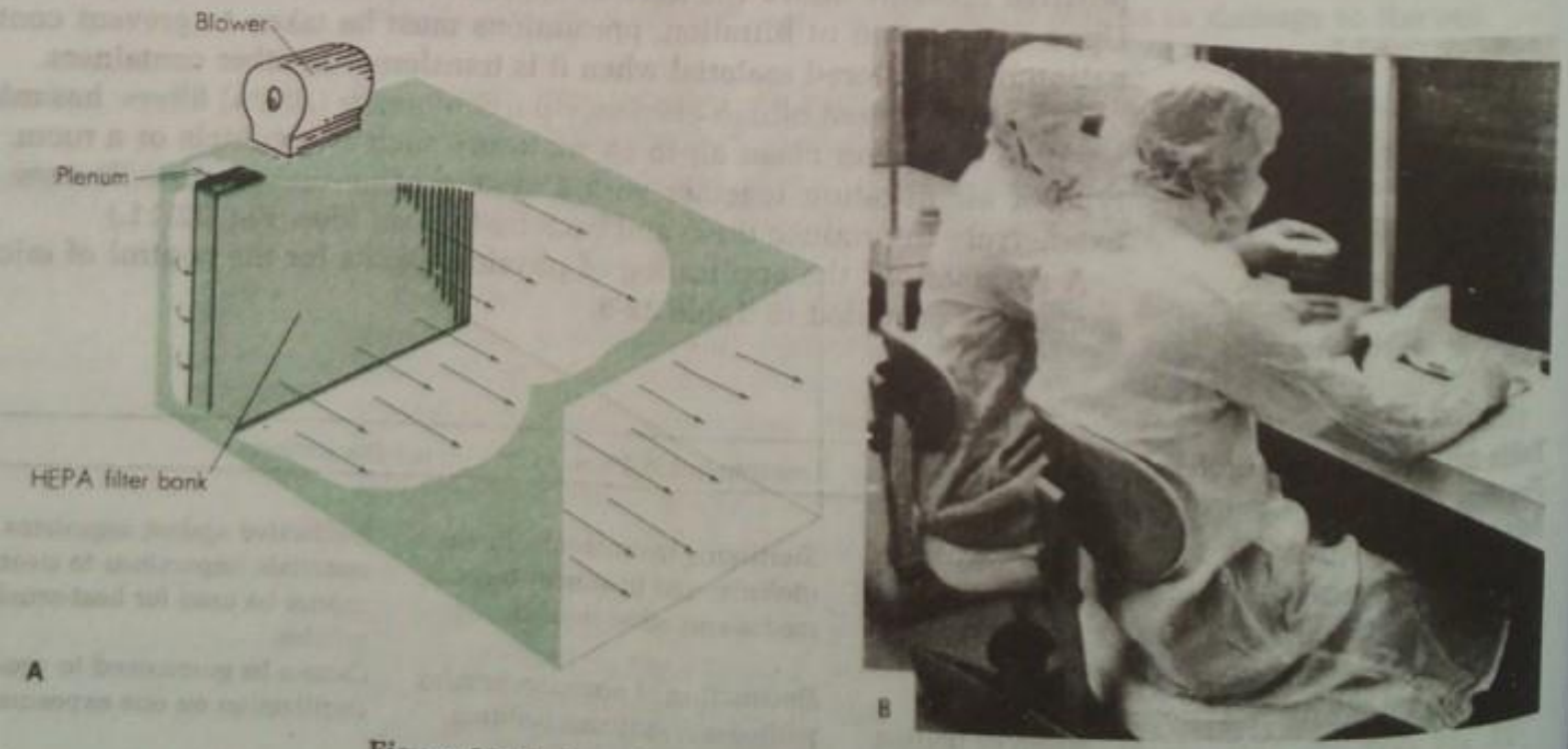


Figure 22-11. Laminar-airflow system. (A) Schematic drawing of horizontal laminar-flow tunnel. Arrows in tunnel denote parallel flow of air through a room. *(Redrawn from M. S. Favero, "Industrial Applications of Laminar Air-flow," in Developments in Industrial Microbiology, American Institute of Biological Sciences, Washington, 1970, vol. 11.)* (B) Laboratory personnel performing sterility test in

1 Describe the death-rate pattern of bacteria when exposed to a lethal agent.
2 Enumerate the conditions which affect the death of microorganisms when they are exposed to an antimicrobial agent.
3 What are the terms by which one can express, quantitatively, the resistance of microorganisms to high temperatures? Distinguish between the meaning of each of these terms.
4 Compare vegetative cells of bacteria with bacterial spores in terms of resistance to heat. What is thought to account for the difference?
5 Describe the process of fractional sterilization, or tyndallization.
6 List several physical agents (or processes) that produce a microbistatic condition.
7 How are microorganisms affected by subzero temperatures?
8 The mechanism of antimicrobial action caused by desiccation is similar to that caused by plasmolysis. Explain why.
9 List several different kinds of radiations that are destructive to microorganisms. Comment on the practical application of each.
10 What is a membrane or molecular filter? How does it differ from older types of bacteriological filters in terms of how it removes microorganisms?
11 What method of sterilization would be appropriate for each of the following?
(*a*) Petri dishes for laboratory use.
(*b*) Water
(*c*) Usual laboratory media, e.g., nutrient agar
(*d*) A dry powder product
(*e*) A heat-labile solution of vitamins
(*f*) A heat-labile antibiotic solution
(*g*) Contaminated hospital linens

See Chap. 23.