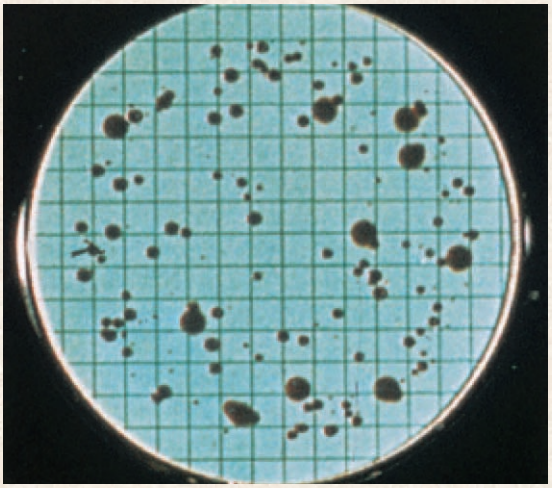
CHAPTER 6

Microbial Growth



Membrane filters are used in counting microorganisms. This membrane has been used to obtain a total bacterial count using an indicator to color colonies for easy counting.

Outline

Concepts

1. Growth is defined as an increase in cellular constituents and may result in an increase in a microorganism's size, population number, or both.
2. When microorganisms are grown in a closed system, population growth remains exponential for only a few generations and then enters a stationary phase due to factors such as nutrient limitation and waste accumulation. In an open system with continual nutrient addition and waste removal, the exponential phase can be maintained for long periods.
3. A wide variety of techniques can be used to study microbial growth by following changes in the total cell number, the population of viable microorganisms, or the cell mass.
4. Water availability, pH, temperature, oxygen concentration, pressure, radiation, and a number of other environmental factors influence microbial growth. Yet many microorganisms, and particularly bacteria, have managed to adapt and flourish under environmental extremes that would destroy most higher organisms.
5. In the natural environment, growth is often severely limited by available nutrient supplies and many other environmental factors.
6. Bacteria can communicate with each other and behave cooperatively using population density–dependent signals.

The paramount evolutionary accomplishment of bacteria as a group is rapid, efficient cell growth in many environments.

—J. L. Ingraham, O. Maaløe, and F. C. Neidhardt

Chapter 5 emphasizes that microorganisms need access to a source of energy and the raw materials essential for the construction of cellular components. All organisms must have carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and a variety of minerals; many also require one or more special growth factors. The cell takes up these substances by membrane transport processes, the most important of which are facilitated diffusion, active transport, and group translocation. Eucaryotic cells also employ endocytosis.

Chapter 6 concentrates more directly on the growth. The nature of growth and the ways in which it can be measured are described first, followed by consideration of continuous culture techniques. An account of the influence of environmental factors on microbial growth completes the chapter.

Growth may be defined as an increase in cellular constituents. It leads to a rise in cell number when microorganisms reproduce by processes like budding or binary fission. In the latter, individual cells enlarge and divide to yield two progeny of approximately equal size. Growth also results when cells simply become longer or larger. If the microorganism is **coenocytic**—that is, a multinucleate organism in which nuclear divisions are not accompanied by cell divisions—growth results in an increase in cell size but not cell number. It is usually not convenient to investigate the growth and reproduction of individual microorganisms because of their small size. Therefore, when studying growth, microbiologists normally follow changes in the total population number. The cell cycle (pp. 87; 285–86)

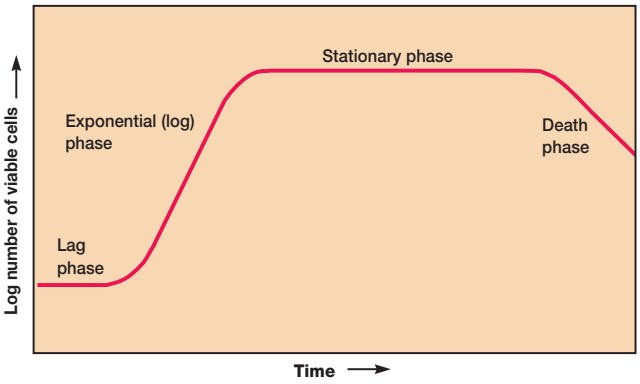


Figure 6.1 Microbial Growth Curve in a Closed System. The four phases of the growth curve are identified on the curve and discussed in the text.

6.1 The Growth Curve

Population growth is studied by analyzing the growth curve of a microbial culture. When microorganisms are cultivated in liquid medium, they usually are grown in a **batch culture** or closed system—that is, they are incubated in a closed culture vessel with a single batch of medium. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases (**figure 6.1**).

Lag Phase

When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs, and therefore this period is called the **lag phase.** Although cell division does not take place right away and there is no net increase in mass, the cell is synthesizing new components. A lag phase prior to the start of cell division can be necessary for a variety of reasons. The cells may be old and depleted of ATP, essential cofactors, and ribosomes; these must be synthesized before growth can begin. The medium may be different from the one the microorganism was growing in previously. Here new enzymes would be needed to use different nutrients. Possibly the microorganisms have been injured and require time to recover. Whatever the causes, eventually the cells retool, replicate their DNA, begin to increase in mass, and finally divide.

The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase.

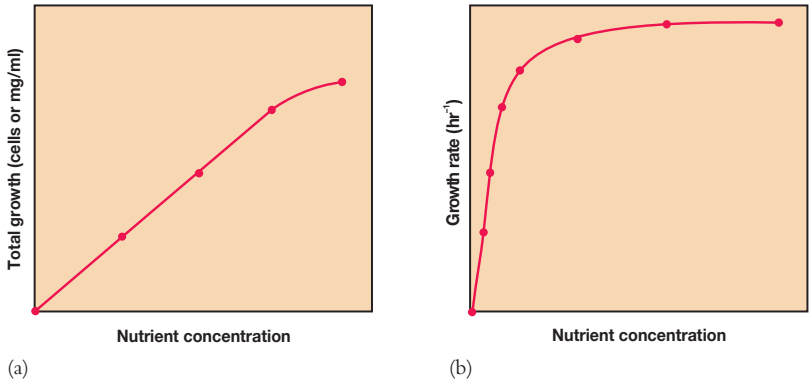


Figure 6.2 Nutrient Concentration and Growth. (**a**) The effect of changes in limiting nutrient concentration on total microbial yield. At sufficiently high concentrations, total growth will plateau. (**b**) The effect on growth rate.

On the other hand, when a young, vigorously growing exponential phase culture is transferred to fresh medium of the same composition, the lag phase will be short or absent.

Exponential Phase

During the **exponential** or **log phase,** microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing. Their rate of growth is constant during the exponential phase; that is, the microorganisms are dividing and doubling in number at regular intervals. Because each individual divides at a slightly different moment, the growth curve rises smoothly rather than in discrete jumps (figure 6.1). The population is most uniform in terms of chemical and physiological properties during this phase; therefore exponential phase cultures are usually used in biochemical and physiological studies.

Exponential growth is **balanced growth.** That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, **unbalanced growth** results. This is growth during which the rates of synthesis of cell components vary relative to one another until a new balanced state is reached. This response is readily observed in a shift-up experiment in which bacteria are transferred from a nutritionally poor medium to a richer one. The cells first construct new ribosomes to enhance their capacity for protein synthesis. This is followed by increases in protein and DNA synthesis. Finally, the expected rise in reproductive rate takes place. Protein and DNA synthesis (sections 11.3 and 12.2)

Unbalanced growth also results when a bacterial population is shifted down from a rich medium to a poor one. The organisms may previously have been able to obtain many cell components directly from the medium. When shifted to a nutritionally inadequate medium, they need time to make the enzymes required for the biosynthesis of unavailable nutrients. Consequently cell division and DNA replication continue after the shift-down, but net protein and RNA synthesis slow. The cells become smaller and reorganize themselves metabolically until they are able to grow again. Then balanced growth is resumed and the culture enters the exponential phase. Regulation of nucleic acid synthesis (pp. 275–83)

These shift-up and shift-down experiments demonstrate that microbial growth is under precise, coordinated control and responds quickly to changes in environmental conditions.

When microbial growth is limited by the low concentration of a required nutrient, the final net growth or yield of cells increases with the initial amount of the limiting nutrient present (**figure 6.2*a***). This is the basis of microbiological assays for vitamins and other growth factors. The rate of growth also increases with nutrient concentration (figure 6.2*b*), but in a hyperbolic manner much like that seen with many enzymes (*see figure 8.17*). The shape of the curve seems to reflect the rate of nutrient uptake by microbial transport proteins. At sufficiently high nutrient levels the transport systems are saturated, and the growth rate does not rise further with increasing nutrient concentration. Microbiological assays (p. 99); Nutrient transport systems (pp. 100–4)

Stationary Phase

Eventually population growth ceases and the growth curve becomes horizontal (figure 6.1). This **stationary phase** usually is attained by bacteria at a population level of around 10^9 cells per ml. Other microorganisms normally do not reach such high population densities, protozoan and algal cultures often having maximum concentrations of about 10^6 cells per ml. Of course final population size depends on nutrient availability and other factors, as well as the type of microorganism being cultured. In the stationary phase the total number of viable microorganisms remains constant. This may result from a balance between cell division and cell death, or the population may simply cease to divide though remaining metabolically active.

Microbial populations enter the stationary phase for several reasons. One obvious factor is nutrient limitation; if an essential nutrient is severely depleted, population growth will slow. Aerobic organisms often are limited by O_2 availability. Oxygen is not very soluble and may be depleted so quickly that only the surface of a culture will have an O_2 concentration adequate for growth. The cells beneath the surface will not be able to grow unless the culture is shaken or aerated in another way. Population growth also may cease due to the accumulation of toxic waste products. This factor seems to limit the growth of many anaerobic cultures (cultures growing in the absence of O_2). For example, streptococci can produce so much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and

growth is inhibited. Streptococcal cultures also can enter the stationary phase due to depletion of their sugar supply. Finally, there is some evidence that growth may cease when a critical population level is reached. Thus entrance into the stationary phase may result from several factors operating in concert.

As we have seen, bacteria in a batch culture may enter stationary phase in response to starvation. This probably often occurs in nature as well because many environments have quite low nutrient levels. Starvation can be a positive experience for bacteria. Many do not respond with obvious morphological changes such as endospore formation, but only decrease somewhat in overall size, often accompanied by protoplast shrinkage and nucleoid condensation. The more important changes are in gene expression and physiology. Starving bacteria frequently produce a variety of **starvation proteins,** which make the cell much more resistant to damage in a variety of ways. They increase peptidoglycan cross-linking and cell wall strength. The Dps (*D*NA-binding *p*rotein from *s*tarved cells) protein protects DNA. Chaperones prevent protein denaturation and renature damaged proteins. As a result of these and many other mechanisms, the starved cells become harder to kill and more resistant to starvation itself, damaging temperature changes, oxidative and osmotic damage, and toxic chemicals such as chlorine. These changes are so effective that some bacteria can survive starvation for years. Clearly, these considerations are of great practical importance in medical and industrial microbiology. There is even evidence that *Salmonella typhimurium* and some other bacterial pathogens become more virulent when starved.

Death Phase

Detrimental environmental changes like nutrient deprivation and the buildup of toxic wastes lead to the decline in the number of viable cells characteristic of the **death phase.** The death of a microbial population, like its growth during the exponential phase, is usually logarithmic (that is, a constant proportion of cells dies every hour). This pattern in viable cell count holds even when the total cell number remains constant because the cells simply fail to lyse after dying. Often the only way of deciding whether a bacterial cell is viable is by incubating it in fresh medium; if it does not grow and reproduce, it is assumed to be dead. That is, death is defined to be the irreversible loss of the ability to reproduce.

Although most of a microbial population usually dies in a logarithmic fashion, the death rate may decrease after the population has been drastically reduced. This is due to the extended survival of particularly resistant cells. For this and other reasons, the death phase curve may be complex.

The Mathematics of Growth

Knowledge of microbial growth rates during the exponential phase is indispensable to microbiologists. Growth rate studies contribute to basic physiological and ecological research and the solution of applied problems in industry. Therefore the quantitative aspects of exponential phase growth will be discussed.

During the exponential phase each microorganism is dividing at constant intervals. Thus the population will double in number during a specific length of time called the **generation time** or **doubling time.** This situation can be illustrated with a simple example. Suppose that a culture tube is inoculated with one cell that divides every 20 minutes (**table 6.1**). The population will be 2 cells after 20 minutes, 4 cells after 40 minutes, and so forth. Because the population is doubling every generation, the increase in population is always 2^n where n is the number of generations. The resulting population increase is exponential or logarithmic (**figure 6.3**).

Table 6.1 An Example of Exponential Growth

Time[a]	Division Number	2^n	Population ($N_0 \times 2^n$)	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

[a]The hypothetical culture begins with one cell having a 20-minute generation time.

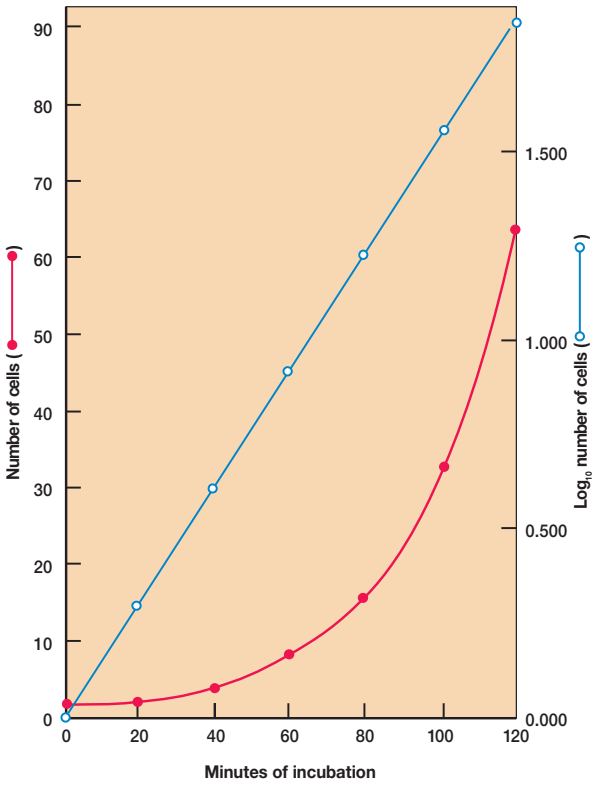


Figure 6.3 **Exponential Microbial Growth.** The data from table 6.1 for six generations of growth are plotted directly (●—●) and in the logarithmic form (○—○). The growth curve is exponential as shown by the linearity of the log plot.

These observations can be expressed as equations for the generation time.

Let N_0 = the initial population number

N_t = the population at time t

n = the number of generations in time t

Then inspection of the results in table 6.1 will show that

$$N_t = N_0 \times 2^n.$$

Solving for n, the number of generations, where all logarithms are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the **mean growth rate constant *(k)*.** This is the number of generations per unit time, often expressed as the generations per hour.

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

The time it takes a population to double in size—that is, the **mean generation time** or mean doubling time (g), can now be calculated. If the population doubles ($t = g$), then

$$N_t = 2\,N_0.$$

Substitute $2N_0$ into the mean growth rate equation and solve for k.

$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The mean generation time is the reciprocal of the mean growth rate constant.

$$g = \frac{1}{k}$$

The mean generation time (g) can be determined directly from a semilogarithmic plot of the growth data (**figure 6.4**) and the growth rate constant calculated from the g value. The generation time also may be calculated directly from the previous equations. For example, suppose that a bacterial population increases from 10^3 cells to 10^9 cells in 10 hours.

$$k = \frac{\log 10^9 - \log 10^3}{(0.301)(10\text{ hr})} = \frac{9 - 3}{3.01\text{ hr}} = 2.0 \text{ generations/hr}$$

$$g = \frac{1}{2.0\text{ gen./hr}} = 0.5 \text{ hr/gen. or } 30 \text{ min/gen.}$$

Generation times vary markedly with the species of microorganism and environmental conditions. They range from less than 10 minutes (0.17 hours) for a few bacteria to several days with some eucaryotic microorganisms (**table 6.2**). Generation times in nature are usually much longer than in culture.

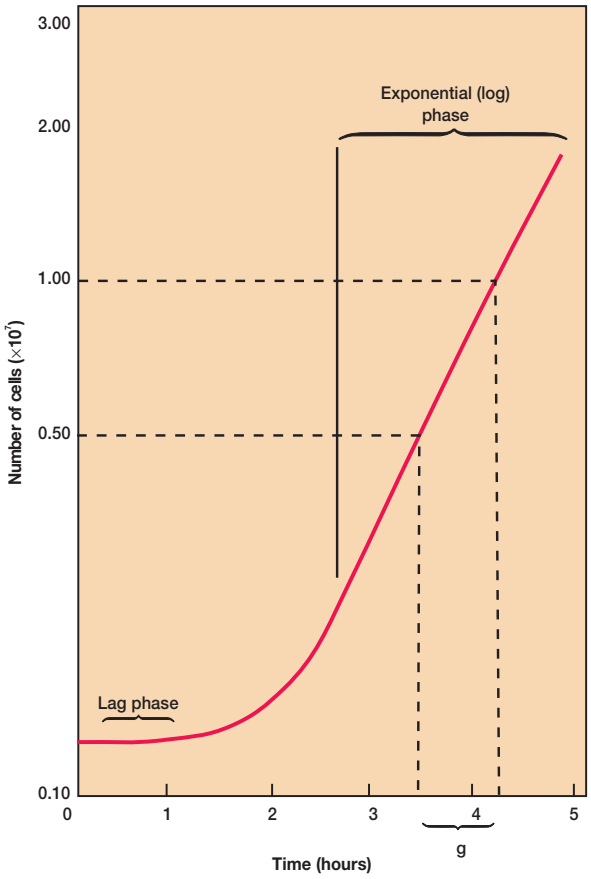


Figure 6.4 Generation Time Determination. The generation time can be determined from a microbial growth curve. The population data are plotted with the logarithmic axis used for the number of cells. The time to double the population number is then read directly from the plot. The log of the population number can also be plotted against time on regular axes.

1. Define growth. Describe the four phases of the growth curve in a closed system and discuss the causes of each.
2. Define balanced growth, unbalanced growth, shift-up experiment, and shift-down experiment.
3. What effect does increasing a limiting nutrient have on the yield of cells and the growth rate?
4. What are the generation or doubling time and the mean growth rate constant? How can they be determined from growth data?

Table 6.2 Generation Times for Selected Microorganisms

Microorganism	Temperature (°C)	Generation Time (Hours)
Bacteria		
Beneckea natriegens	37	0.16
Escherichia coli	40	0.35
Bacillus subtilis	40	0.43
Staphylococcus aureus	37	0.47
Pseudomonas aeruginosa	37	0.58
Clostridium botulinum	37	0.58
Rhodospirillum rubrum	25	4.6–5.3
Anabaena cylindrica	25	10.6
Mycobacterium tuberculosis	37	≈12
Treponema pallidum	37	33
Algae		
Scenedesmus quadricauda	25	5.9
Chlorella pyrenoidosa	25	7.75
Asterionella formosa	20	9.6
Euglena gracilis	25	10.9
Ceratium tripos	20	82.8
Protozoa		
Tetrahymena geleii	24	2.2–4.2
Leishmania donovani	26	10–12
Paramecium caudatum	26	10.4
Acanthamoeba castellanii	30	11–12
Giardia lamblia	37	18
Fungi		
Saccharomyces cerevisiae	30	2
Monilinia fructicola	25	30

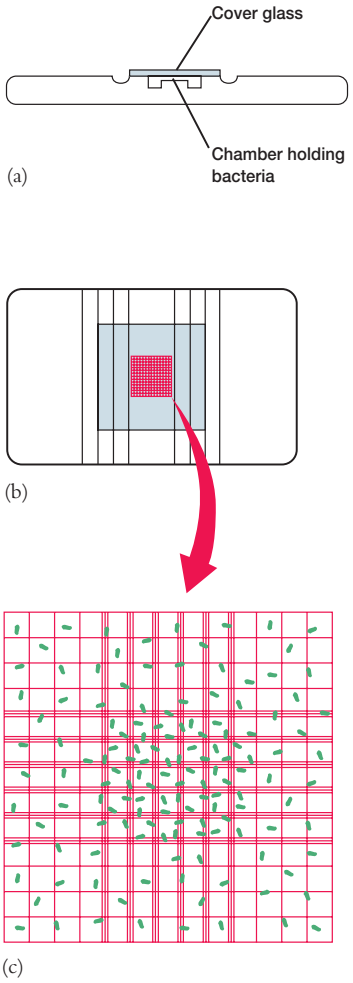


Figure 6.5 **The Petroff-Hausser Counting Chamber.** (**a**) Side view of the chamber showing the cover glass and the space beneath it that holds a bacterial suspension. (**b**) A top view of the chamber. The grid is located in the center of the slide. (**c**) An enlarged view of the grid. The bacteria in several of the central squares are counted, usually at ×400 to ×500 magnification. The average number of bacteria in these squares is used to calculate the concentration of cells in the original sample. Since there are 25 squares covering an area of 1 mm^2, the total number of bacteria in 1 mm^2 of the chamber is (number/square)(25 squares). The chamber is 0.02 mm deep and therefore,

$$\text{bacteria/mm}^3 = (\text{bacteria/square})(25\ \text{squares})(50).$$

The number of bacteria per cm^3 is 10^3 times this value. For example, suppose the average count per square is 28 bacteria:

$$\text{bacteria/cm}^3 = (28\ \text{bacteria})\ (25\ \text{squares})(50)(10^3) = 3.5 \times 10^7.$$

6.2 Measurement of Microbial Growth

There are many ways to measure microbial growth to determine growth rates and generation times. Either population mass or number may be followed because growth leads to increases in both. The most commonly employed techniques for growth measurement are examined briefly and the advantages and disadvantages of each noted. No single technique is always best; the most appropriate approach will depend on the experimental situation.

Measurement of Cell Numbers

The most obvious way to determine microbial numbers is through direct counting. Using a counting chamber is easy, inexpensive, and relatively quick; it also gives information about the size and morphology of microorganisms. Petroff-Hausser counting chambers can be used for counting procaryotes; hemocytometers can be used for both procaryotes and eucaryotes. Procaryotes are more easily counted in these chambers if they are stained, or when a phase-contrast or a fluorescence microscope is employed. These specially designed slides have chambers of known depth with an etched grid on the chamber bottom (**figure 6.5**). The number of microorganisms in a sample can be calculated by taking into account the chamber's volume and any

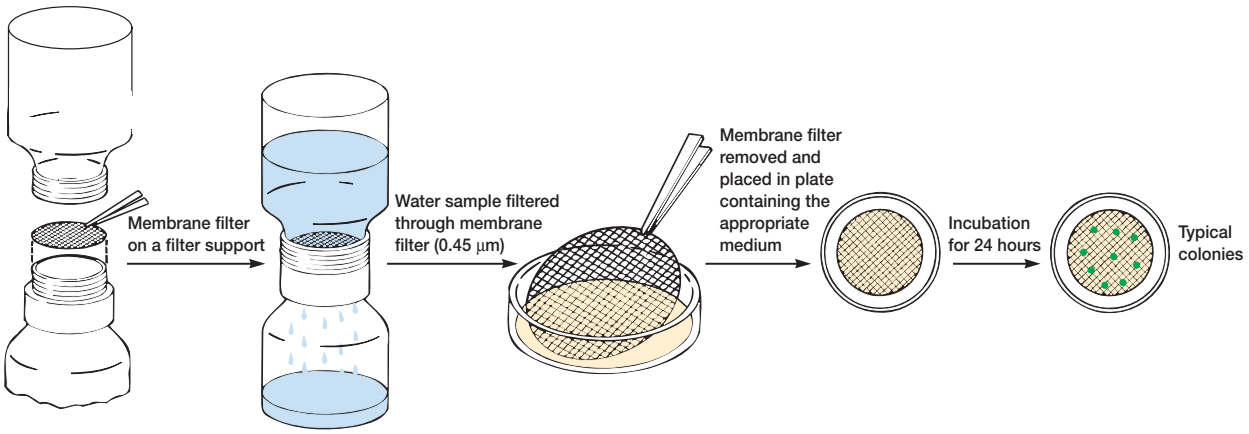


Figure 6.6 The Membrane Filtration Procedure. Membranes with different pore sizes are used to trap different microorganisms. Incubation times for membranes also vary with the medium and microorganism.

sample dilutions required. There are some disadvantages to the technique. The microbial population must be fairly large for accuracy because such a small volume is sampled. It is also difficult to distinguish between living and dead cells in counting chambers without special techniques.

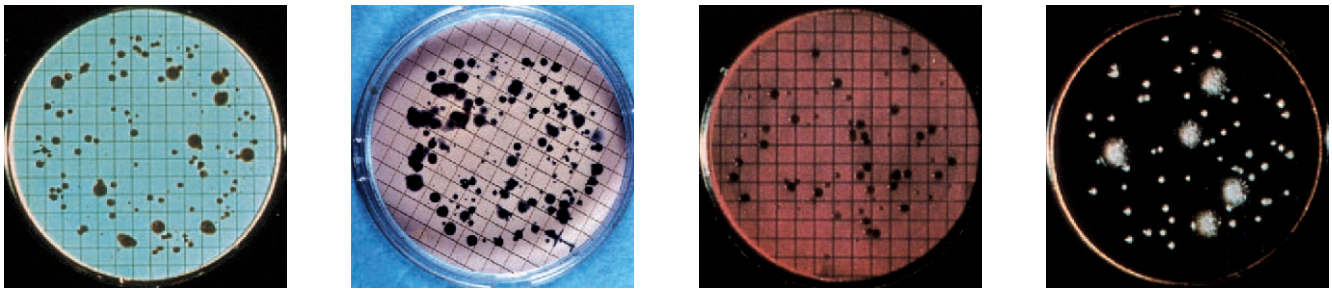
Larger microorganisms such as protozoa, algae, and nonfilamentous yeasts can be directly counted with electronic counters such as the Coulter Counter. The microbial suspension is forced through a small hole or orifice. An electrical current flows through the hole, and electrodes placed on both sides of the orifice measure its electrical resistance. Every time a microbial cell passes through the orifice, electrical resistance increases (or the conductivity drops) and the cell is counted. The Coulter Counter gives accurate results with larger cells and is extensively used in hospital laboratories to count red and white blood cells. It is not as useful in counting bacteria because of interference by small debris particles, the formation of filaments, and other problems.

Counting chambers and electronic counters yield counts of all cells, whether alive or dead. There are also several viable counting techniques, procedures specific for cells able to grow and reproduce. In most viable counting procedures, a diluted sample of bacteria or other microorganisms is dispersed over a solid agar surface. Each microorganism or group of microorganisms develops into a distinct colony. The original number of viable microorganisms in the sample can be calculated from the number of colonies formed and the sample dilution. For example, if 1.0 ml of a 1×10^{-6} dilution yielded 150 colonies, the original sample contained around 1.5×10^{8} cells per ml. Usually the count is made more accurate by use of a special colony counter. In this way the spread-plate and pour-plate techniques may be used to find the number of microorganisms in a sample.

Plating techniques are simple, sensitive, and widely used for viable counts of bacteria and other microorganisms in samples of food, water, and soil. Several problems, however, can lead to inaccurate counts. Low counts will result if clumps of cells are not broken up and the microorganisms well dispersed. Because it is not possible to be absolutely certain that each colony arose from an individual cell, the results are often expressed in terms of **colony forming units (CFU)** rather than the number of microorganisms. The samples should yield between 30 and 300 colonies for best results. Of course the counts will also be low if the agar medium employed cannot support growth of all the viable microorganisms present. The hot agar used in the pour-plate technique may injure or kill sensitive cells; thus spread plates sometimes give higher counts than pour plates. Spread-plate and pour-plate techniques (pp. 106–8)

Microbial numbers are frequently determined from counts of colonies growing on special membrane filters having pores small enough to trap bacteria. In the membrane filter technique, a sample is drawn through a special **membrane filter** (**figure 6.6**). The filter is then placed on an agar medium or on a pad soaked with liquid media and incubated until each cell forms a separate colony. A colony count gives the number of microorganisms in the filtered sample, and special media can be used to select for specific microorganisms (**figure 6.7**). This technique is especially useful in analyzing aquatic samples. Analysis of water purity (pp. 653–57)

Membrane filters also are used to count bacteria directly. The sample is first filtered through a black polycarbonate membrane filter to provide a good background for observing fluorescent objects. The bacteria then are stained with a fluorescent dye such as acridine orange or DAPI and observed microscopically. Acridine orange–stained microorganisms glow orange or green and are easily counted with an epifluorescence microscope (*see section 2.2*). Usually the counts obtained with this approach are much higher than those from culture techniques because some of the bacteria are dead. Commercial kits that use fluorescent reagents to stain live and dead cells differently are now available. This makes it possible to directly count the number of live and dead microorganisms in a sample (*see figure 2.13*d).



(a) (b) (c) (d)

Figure 6.7 Colonies on Membrane Filters. Membrane-filtered samples grown on a variety of media. (**a**) Standard nutrient media for a total bacterial count. An indicator colors colonies red for easy counting. (**b**) Fecal coliform medium for detecting fecal coliforms that form blue colonies. (**c**) m-Endo agar for detecting *E. coli* and other coliforms that produce colonies with a green sheen. (**d**) Wort agar for the culture of yeasts and molds.

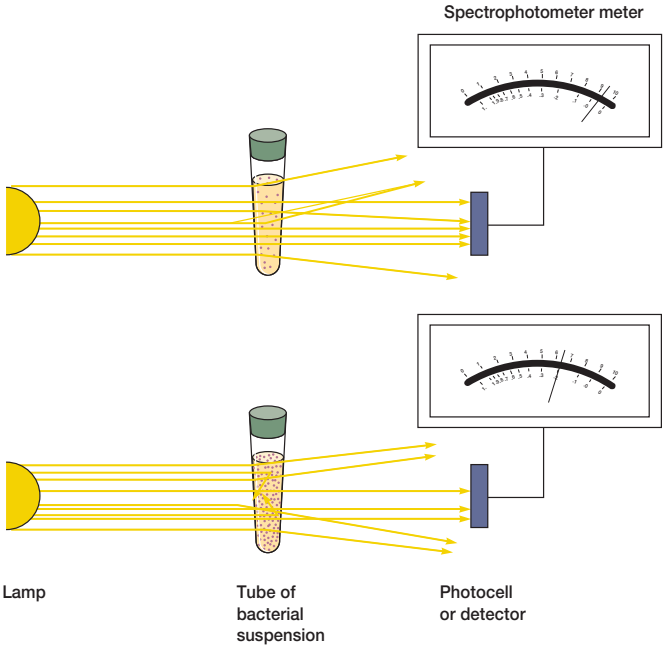


Figure 6.8 Turbidity and Microbial Mass Measurement. Determination of microbial mass by measurement of light absorption. As the population and turbidity increase, more light is scattered and the absorbance reading given by the spectrophotometer increases. The spectrophotometer meter has two scales. The bottom scale displays absorbance and the top scale, percent transmittance. Absorbance increases as percent transmittance decreases.

Measurement of Cell Mass

Increases in the total cell mass, as well as in cell numbers, accompany population growth. Therefore techniques for measuring changes in cell mass can be used in following growth. The most direct approach is the determination of microbial dry weight. Cells growing in liquid medium are collected by centrifugation, washed, dried in an oven, and weighed. This is an especially useful technique for measuring the growth of fungi. It is time consuming, however, and not very sensitive. Because bacteria weigh so little, it may be necessary to centrifuge several hundred milliliters of culture to collect a sufficient quantity.

More rapid, sensitive techniques depend on the fact that microbial cells scatter light striking them. Because microbial cells in a population are of roughly constant size, the amount of scattering is directly proportional to the biomass of cells present and indirectly related to cell number. When the concentration of bacteria reaches about 10 million cells (10^7) per ml, the medium appears slightly cloudy or turbid. Further increases in concentration result in greater turbidity and less light is transmitted through the medium. The extent of light scattering can be measured by a spectrophotometer and is almost linearly related to bacterial concentration at low absorbance levels (**figure 6.8**). Thus population growth can be easily measured spectrophotometrically as long as the population is high enough to give detectable turbidity.

If the amount of a substance in each cell is constant, the total quantity of that cell constituent is directly related to the total

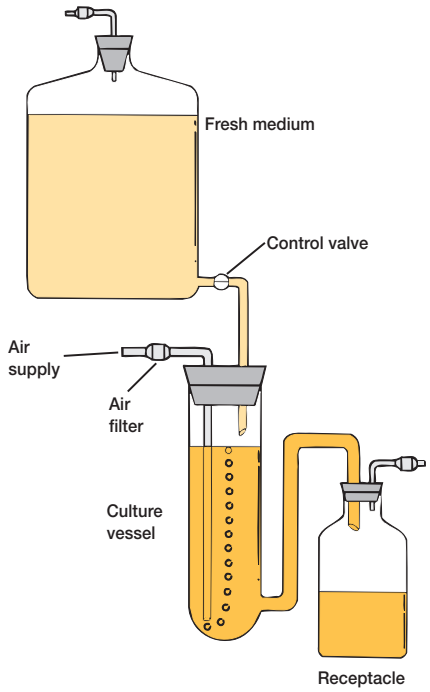


Figure 6.9 A Continuous Culture System: The Chemostat. Schematic diagram of the system. The fresh medium contains a limiting amount of an essential nutrient. Growth rate is determined by the rate of flow of medium through the culture vessel.

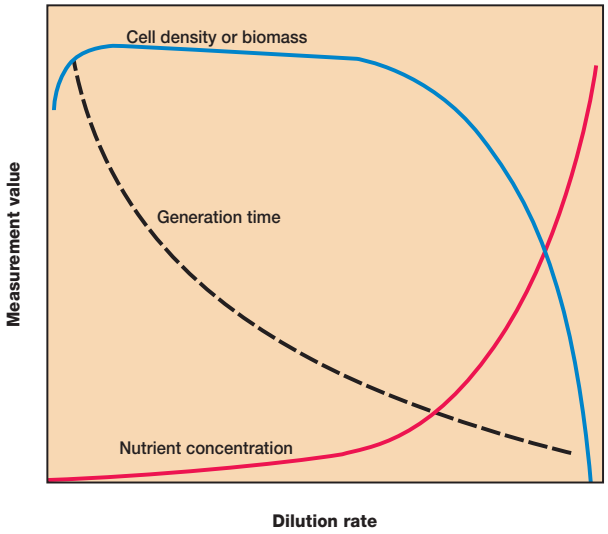


Figure 6.10 Chemostat Dilution Rate and Microbial Growth. The effects of changing the dilution rate in a chemostat.

microbial cell mass. For example, a sample of washed cells collected from a known volume of medium can be analyzed for total protein or nitrogen. An increase in the microbial population will be reflected in higher total protein levels. Similarly, chlorophyll determinations can be used to measure algal populations, and the quantity of ATP can be used to estimate the amount of living microbial mass.

1. Briefly describe each technique by which microbial population numbers may be determined and give its advantages and disadvantages.
2. Why are plate count results often expressed as colony forming units?

6.3 The Continuous Culture of Microorganisms

Up to this point the focus has been on closed systems called batch cultures in which nutrient supplies are not renewed nor wastes removed. Exponential growth lasts for only a few generations and soon the stationary phase is reached. However, it is possible to grow microorganisms in an open system, a system with constant environmental conditions maintained through continual provision of nutrients and removal of wastes. These conditions are met in the laboratory by a **continuous culture system.** A microbial population can be maintained in the exponential growth phase and at a constant biomass concentration for extended periods in a continuous culture system.

The Chemostat

Two major types of continuous culture systems commonly are used: (1) chemostats and (2) turbidostats. A **chemostat** is constructed so that sterile medium is fed into the culture vessel at the same rate as the media containing microorganisms is removed (**figure 6.9**). The culture medium for a chemostat possesses an essential nutrient (e.g., an amino acid) in limiting quantities. Because of the presence of a limiting nutrient, the growth rate is determined by the rate at which new medium is fed into the growth chamber, and the final cell density depends on the concentration of the limiting nutrient. The rate of nutrient exchange is expressed as the dilution rate (D), the rate at which medium flows through the culture vessel relative to the vessel volume, where f is the flow rate (ml/hr) and V is the vessel volume (ml).

$$D = f/V$$

For example, if f is 30 ml/hr and V is 100 ml, the dilution rate is 0.30 hr^{-1}.

Both the microbial population level and the generation time are related to the dilution rate (**figure 6.10**). The microbial population density remains unchanged over a wide range of dilution rates. The generation time decreases (i.e., the growth rate rises) as the dilution rate increases. The limiting nutrient will be almost

completely depleted under these balanced conditions. If the dilution rate rises too high, the microorganisms can actually be washed out of the culture vessel before reproducing because the dilution rate is greater than the maximum growth rate. The limiting nutrient concentration rises at higher dilution rates because fewer microorganisms are present to use it.

At very low dilution rates, an increase in *D* causes a rise in both cell density and the growth rate. This is because of the effect of nutrient concentration on the growth rate, sometimes called the Monod relationship (figure 6.2*b*). Only a limited supply of nutrient is available at low dilution rates. Much of the available energy must be used for cell maintenance, not for growth and reproduction. As the dilution rate increases, the amount of nutrients and the resulting cell density rise because energy is available for both maintenance and growth. The growth rate increases when the total available energy exceeds the **maintenance energy.**

The Turbidostat

The second type of continuous culture system, the **turbidostat,** has a photocell that measures the absorbance or turbidity of the culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity or cell density. The turbidostat differs from the chemostat in several ways. The dilution rate in a turbidostat varies rather than remaining constant, and its culture medium lacks a limiting nutrient. The turbidostat operates best at high dilution rates; the chemostat is most stable and effective at lower dilution rates.

Continuous culture systems are very useful because they provide a constant supply of cells in exponential phase and growing at a known rate. They make possible the study of microbial growth at very low nutrient levels, concentrations close to those present in natural environments. These systems are essential for research in many areas—for example, in studies on interactions between microbial species under environmental conditions resembling those in a freshwater lake or pond. Continuous systems also are used in food and industrial microbiology.

1. How does an open system differ from a closed culture system or batch culture?
2. Describe how the two different kinds of continuous culture systems, the chemostat and turbidostat, operate.
3. What is the dilution rate? What is maintenance energy?

6.4 The Influence of Environmental Factors on Growth

As we have seen (pp. 114–15), microorganisms must be able to respond to variations in nutrient levels, and particularly to nutrient limitation. The growth of microorganisms also is greatly affected by the chemical and physical nature of their surroundings. An understanding of environmental influences aids in the control of microbial growth and the study of the ecological distribution of microorganisms.

The ability of some microorganisms to adapt to extreme and inhospitable environments is truly remarkable. Procaryotes are present anywhere life can exist. Many habitats in which procaryotes thrive would kill most other organisms. Procaryotes such as *Bacillus infernus* even seem able to live over 1.5 miles below the Earth's surface, without oxygen and at temperatures above 60°C. Microorganisms that grow in such harsh conditions are often called **extremophiles.**

In this section we shall briefly review how some of the most important environmental factors affect microbial growth. Major emphasis will be given to solutes and water activity, pH, temperature, oxygen level, pressure, and radiation. **Table 6.3** summarizes the way in which microorganisms are categorized in terms of their response to these factors.

Solutes and Water Activity

Because a selectively permeable plasma membrane separates microorganisms from their environment, they can be affected by changes in the osmotic concentration of their surroundings. If a microorganism is placed in a hypotonic solution (one with a lower osmotic concentration), water will enter the cell and cause it to burst unless something is done to prevent the influx. The osmotic concentration of the cytoplasm can be reduced by use of inclusion bodies (*see pp. 49–52*). Procaryotes also can contain pressure-sensitive channels that open to allow solute escape when the osmolarity of the environment becomes much lower than that of the cytoplasm.

Most bacteria, algae, and fungi have rigid cell walls that maintain the shape and integrity of the cell. When microorganisms with rigid cell walls are placed in a hypertonic environment, water leaves and the plasma membrane shrinks away from the wall, a process known as plasmolysis. This dehydrates the cell and may damage the plasma membrane; the cell usually becomes metabolically inactive and ceases to grow.

Many microorganisms keep the osmotic concentration of their protoplasm somewhat above that of the habitat by the use of compatible solutes, so that the plasma membrane is always pressed firmly against their cell wall. **Compatible solutes** are solutes that are compatible with metabolism and growth when at high intracellular concentrations. Most procaryotes increase their internal osmotic concentration in a hypertonic environment through the synthesis or uptake of choline, betaine, proline, glutamic acid, and other amino acids; elevated levels of potassium ions are also involved to some extent. Algae and fungi employ sucrose and polyols—for example, arabitol, glycerol, and mannitol—for the same purpose. Polyols and amino acids are ideal solutes for this function because they normally do not disrupt enzyme structure and function. A few procaryotes like *Halobacterium salinarium* raise their osmotic concentration with potassium ions (sodium ions are also elevated but not as much as potassium). *Halobacterium*'s enzymes have been altered so that they actually require high salt concentrations for normal activity (*see section 20.3*). Since protozoa do not have a cell wall, they must use contractile vacuoles (*see figure 27.3*) to eliminate excess water when living in hypotonic environments. Osmosis and the protective function of the cell wall (p. 61)

Table 6.3 Microbial Responses to Environmental Factors

Descriptive Term	Definition	Representative Microorganisms
Solute and Water Activity		
Osmotolerant	Able to grow over wide ranges of water activity or osmotic concentration	*Staphylococcus aureus, Saccharomyces rouxii*
Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	*Halobacterium, Dunaliella, Ectothiorhodospira*
pH		
Acidophile	Growth optimum between pH 0 and 5.5	*Sulfolobus, Picrophilus, Ferroplasma, Acontium, Cyanidium caldarium*
Neutrophile	Growth optimum between pH 5.5 and 8.0	*Escherichia, Euglena, Paramecium*
Alkalophile	Growth optimum between pH 8.5 and 11.5	*Bacillus alcalophilus, Natronobacterium*
Temperature		
Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	*Bacillus psychrophilus, Chlamydomonas nivalis*
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	*Listeria monocytogenes, Pseudomonas fluorescens*
Mesophile	Has growth optimum around 20–45°C	*Escherichia coli, Neisseria gonorrhoeae, Trichomonas vaginalis*
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	*Bacillus stearothermophilus, Thermus aquaticus, Cyanidium caldarium, Chaetomium thermophile*
Hyperthermophile	Has an optimum between 80 and about 113°C	*Sulfolobus, Pyrococcus, Pyrodictium*
Oxygen Concentration		
Obligate aerobe	Completely dependent on atmospheric O_2 for growth.	*Micrococcus luteus, Pseudomonas, Mycobacterium;* most algae, fungi, and protozoa
Facultative anaerobe	Does not require O_2 for growth, but grows better in its presence.	*Escherichia, Enterococcus, Saccharomyces cerevisiae*
Aerotolerant anaerobe	Grows equally well in presence or absence of O_2	*Streptococcus pyogenes*
Obligate anaerobe	Does not tolerate O_2 and dies in its presence.	*Clostridium, Bacteroides, Methanobacterium, Trepomonas agilis*
Microaerophile	Requires O_2 levels below 2–10% for growth and is damaged by atmospheric O_2 (20%).	*Campylobacter, Spirillum volutans, Treponema pallidum*
Pressure		
Barophilic	Growth more rapid at high hydrostatic pressures.	*Photobacterium profundum, Shewanella benthica, Methanococcus jannaschii*

The amount of water available to microorganisms can be reduced by interaction with solute molecules (the osmotic effect) or by adsorption to the surfaces of solids (the matric effect). Because the osmotic concentration of a habitat has such profound effects on microorganisms, it is useful to be able to express quantitatively the degree of water availability. Microbiologists generally use **water activity (a_w)** for this purpose (water availability also may be expressed as water potential, which is related to a_w). The water activity of a solution is 1/100 the relative humidity of the solution (when expressed as a percent). It is also equivalent to the ratio of the solution's vapor pressure (P_{soln}) to that of pure water (P_{water}).

$$a_w = \frac{P_{soln}}{P_{water}}$$

The water activity of a solution or solid can be determined by sealing it in a chamber and measuring the relative humidity after the system has come to equilibrium. Suppose after a sample is treated in this way, the air above it is 95% saturated—that is, the air contains 95% of the moisture it would have when equilibrated at the same temperature with a sample of pure water. The relative humidity would be 95% and the sample's water activity, 0.95. Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, its a_w is low.

Microorganisms differ greatly in their ability to adapt to habitats with low water activity (**table 6.4**). A microorganism must expend extra effort to grow in a habitat with a low a_w value because it must maintain a high internal solute concentration to retain water. Some microorganisms can do this and are **osmotolerant;** they will grow over wide ranges of water activity or osmotic concentration. For example, *Staphylococcus aureus* can be cultured in media containing any sodium chloride concentration up to about 3 M. It is well adapted for growth on the skin. The yeast *Saccharomyces rouxii* will grow in sugar solutions with a_w values as low as 0.6. The alga *Dunaliella viridis* tolerates sodium chloride concentrations from 1.7 M to a saturated solution.

Although a few microorganisms are truly osmotolerant, most only grow well at water activities around 0.98 (the approximate a_w for seawater) or higher. This is why drying food or adding large quantities of salt and sugar is so effective in preventing food spoilage. As table 6.4 shows, many fungi are osmotolerant and thus particularly important in the spoilage of salted or dried foods. Food spoilage (pp. 966–69)

Table 6.4 Approximate Lower a_w Limits for Microbial Growth

Water Activity	Environment	Bacteria	Fungi	Algae
1.00—Pure water	Blood, Plant wilt, Seawater { Vegetables, meat, fruit	Most gram-negative nonhalophiles		
0.95	Bread	Most gram-positive rods	*Basidiomycetes*	Most algae
0.90	Ham	Most cocci, *Bacillus*	*Fusarium* *Mucor, Rhizopus* Ascomycetous yeasts	
0.85	Salami	*Staphylococcus*	*Saccharomyces rouxii* (in salt)	
0.80	Preserves		*Penicillium*	
0.75	Salt lakes Salted fish	*Halobacterium* *Actinospora*	*Aspergillus*	*Dunaliella*
0.70	Cereals, candy, dried fruit		*Aspergillus*	
0.60	Chocolate Honey Dried milk		*Saccharomyces rouxii* (in sugars) *Xeromyces bisporus*	
0.55—DNA disordered				

Adapted from A. D. Brown, "Microbial Water Stress," in *Bacteriological Reviews,* 40(4):803–846 1976. Copyright © 1976 by the American Society for Microbiology. Reprinted by permission.

Halophiles have adapted so completely to hypertonic, saline conditions that they require high levels of sodium chloride to grow, concentrations between about 2.8 M and saturation (about 6.2 M) for extreme halophilic bacteria. The archaeon *Halobacterium* can be isolated from the Dead Sea (a salt lake between Israel and Jordan and the lowest lake in the world), the Great Salt Lake in Utah, and other aquatic habitats with salt concentrations approaching saturation. *Halobacterium* and other extremely halophilic bacteria have significantly modified the structure of their proteins and membranes rather than simply increasing the intracellular concentrations of solutes, the approach used by most osmotolerant microorganisms. These extreme halophiles accumulate enormous quantities of potassium in order to remain hypertonic to their environment; the internal potassium concentration may reach 4 to 7 M. The enzymes, ribosomes, and transport proteins of these bacteria require high levels of potassium for stability and activity. In addition, the plasma membrane and cell wall of *Halobacterium* are stabilized by high concentrations of sodium ion. If the sodium concentration decreases too much, the wall and plasma membrane literally disintegrate. Extreme halophilic bacteria have successfully adapted to environmental conditions that would destroy most organisms. In the process they have become so specialized that they have lost ecological flexibility and can prosper only in a few extreme habitats. The halobacteria (section 20.3)

1. How do microorganisms adapt to hypotonic and hypertonic environments? What is plasmolysis?
2. Define water activity and briefly describe how it can be determined.
3. Why is it difficult for microorganisms to grow at low a_w values?
4. What are halophiles and why does *Halobacterium* require sodium and potassium ions?

pH

pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration (expressed in terms of molarity).

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

The pH scale extends from pH 0.0 (1.0 M H^+) to pH 14.0 (1.0×10^{-14} M H^+), and each pH unit represents a tenfold change in hydrogen ion concentration. **Figure 6.11** shows that the habitats in which microorganisms grow vary widely—from pH 1 to 2 at the acid end to alkaline lakes and soil that may have pH values between 9 and 10.

It is not surprising that pH dramatically affects microbial growth. Each species has a definite pH growth range and pH growth optimum. **Acidophiles** have their growth optimum between pH 0 and 5.5; **neutrophiles,** between pH 5.5 and 8.0; and **alkalophiles** prefer the pH range of 8.5 to 11.5. Extreme alkalophiles have growth optima at pH 10 or higher. In general, different microbial groups have characteristic pH preferences. Most bacteria and protozoa are neutrophiles. Most fungi prefer slightly acid surroundings, about pH 4 to 6; algae also seem to favor slight acidity. There are many exceptions to these generalizations. For example, the alga *Cyanidium caldarium* and the archaeon *Sulfolobus acidocaldarius* are common inhabitants of acidic hot springs; both grow well around pH 1 to 3 and at high temperatures. The Archaea *Ferroplasma acidarmanus* and *Picrophilus oshimae* can actually grow at pH 0, or very close to it.

Although microorganisms will often grow over wide ranges of pH and far from their optima, there are limits to their tolerance. Drastic variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of

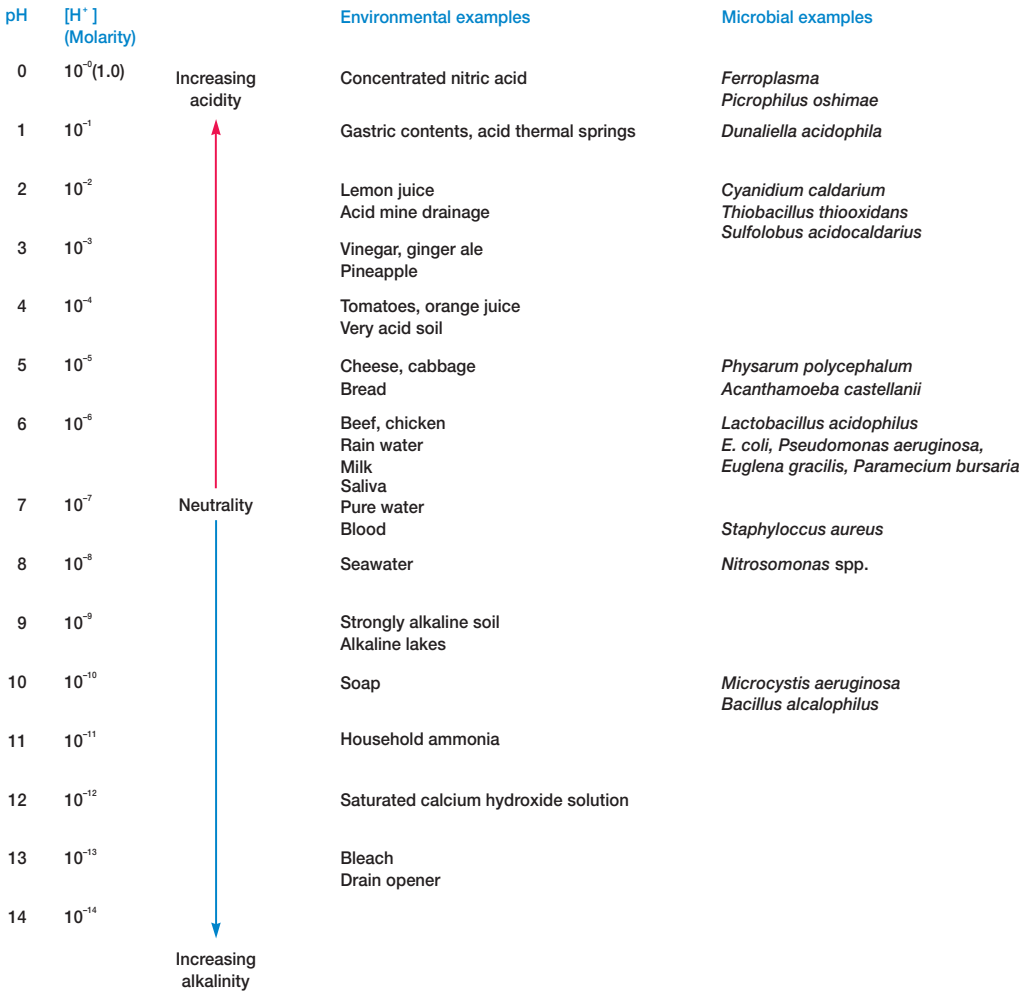


Figure 6.11 **The pH Scale.** The pH scale and examples of substances with different pH values. The microorganisms are placed at their growth optima.

enzymes and membrane transport proteins. Procaryotes die if the internal pH drops much below 5.0 to 5.5. Changes in the external pH also might alter the ionization of nutrient molecules and thus reduce their availability to the organism.

Several mechanisms for the maintenance of a neutral cytoplasmic pH have been proposed. The plasma membrane may be relatively impermeable to protons. Neutrophiles appear to exchange potassium for protons using an antiport transport system (*p. 102*). Extreme alkalophiles like *Bacillus alcalophilus* maintain their internal pH closer to neutrality by exchanging internal sodium ions for external protons. Internal buffering also may contribute to pH homeostasis.

Microorganisms often must adapt to environmental pH changes to survive. In bacteria, potassium/proton and sodium/proton antiport systems probably correct small variations in pH. If the pH becomes too acidic, other mechanisms come into play. When the pH drops below about 5.5 to 6.0, *Salmonella typhimurium* and *E. coli* synthesize an array of new proteins as part of what has been called their acidic tolerance response. A proton-translocating ATPase contributes to this protective response, either by making more ATP or by pumping protons out of the cell. If the external pH decreases to 4.5 or lower, chaperones such as acid shock proteins and heat shock proteins are synthesized (*see pp. 272–74*). Presumably these prevent the acid denaturation of proteins and aid in the refolding of denatured proteins.

Microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste products. Fermentative microorganisms form organic acids from carbohydrates, whereas chemolithotrophs like *Thiobacillus* oxidize reduced sulfur components to sulfuric acid. Other microorganisms make their environment more alkaline by generating ammonia through amino acid degradation. Microbial fermentations (pp. 179–81); Sulfur-oxidizing bacteria (pp. 496–98)

Buffers often are included in media to prevent growth inhibition by large pH changes. Phosphate is a commonly used buffer and a good example of buffering by a weak acid ($H_2PO_4^-$) and its conjugate base (HPO_4^{2-}).

$$H^+ + HPO_4^{2-} \longrightarrow H_2PO_4^-$$

$$OH^- + H_2PO_4^- \longrightarrow HPO_4^{2-} + HOH$$

If protons are added to the mixture, they combine with the salt form to yield a weak acid. An increase in alkalinity is resisted because the weak acid will neutralize hydroxyl ions through proton donation to give water. Peptides and amino acids in complex media also have a strong buffering effect.

Temperature

Environmental temperature profoundly affects microorganisms, like all other organisms. Indeed, microorganisms are particularly susceptible because they are usually unicellular and their temperature varies with that of the external environment. For these reasons, microbial cell temperature directly reflects that of the cell's surroundings. A most important factor influencing the effect of temperature on growth is the temperature sensitivity of enzyme-catalyzed reactions. At low temperatures a temperature rise increases the growth rate because the velocity of an enzyme-catalyzed reaction, like that of any chemical reaction, will roughly double for every 10°C rise in temperature. Because the rate of each reaction increases, metabolism as a whole is more active at higher temperatures, and the microorganism grows faster. Beyond a certain point further increases actually slow growth, and sufficiently high temperatures are lethal. High temperatures damage microorganisms by denaturing enzymes, transport carriers, and other proteins. Microbial membranes are also disrupted by temperature extremes; the lipid bilayer simply melts and disintegrates. Thus, although functional enzymes operate more rapidly at higher temperatures, the microorganism may be damaged to such an extent that growth is inhibited because the damage cannot be repaired. At very low temperatures, membranes solidify and enzymes don't work rapidly. In summary, when organisms are above the optimum temperature, both function and cell structure are affected. If temperatures are very low, function is affected but not necessarily cell chemical composition and structure. The temperature dependence of enzyme activity (pp. 163–64)

Because of these opposing temperature influences, microbial growth has a fairly characteristic temperature dependence with distinct **cardinal temperatures**—minimum, optimum, and maximum growth temperatures (**figure 6.12**). Although the shape of the temperature dependence curve can vary, the temperature optimum is always closer to the maximum than to the minimum. The cardinal temperatures for a particular species are not rigidly fixed but often depend to some extent on other environmental factors such as pH and the available nutrients. For example, *Crithidia fasciculata,* a flagellated protozoan living in the gut of mosquitos, will grow in a simple medium at 22 to 27°C. However, it cannot be cultured at 33 to 34°C without the addition of extra metals, amino acids, vitamins, and lipids.

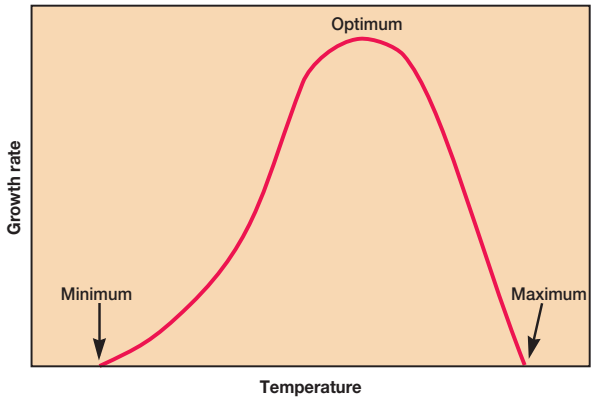


Figure 6.12 **Temperature and Growth.** The effect of temperature on growth rate.

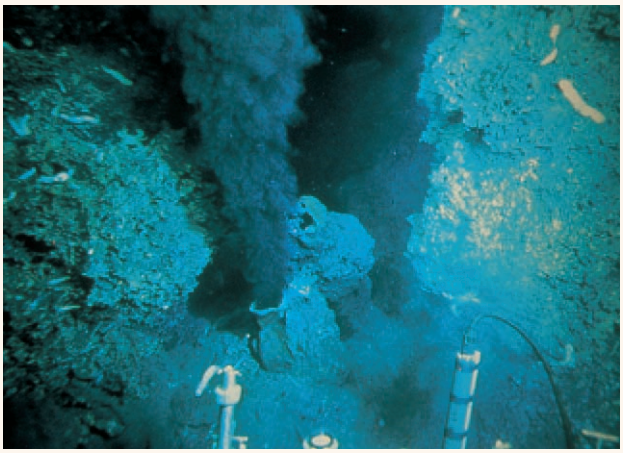
The cardinal temperatures vary greatly between microorganisms (table 6.5). Optima normally range from 0°C to as high as 75°C, whereas microbial growth occurs at temperatures extending from −20°C to over 100°C. The major factor determining this growth range seems to be water. Even at the most extreme temperatures, microorganisms need liquid water to grow. The growth temperature range for a particular microorganism usually spans about 30 degrees. Some species (e.g., *Neisseria gonorrhoeae*) have a small range; others, like *Enterococcus faecalis,* will grow over a wide range of temperatures. The major microbial groups differ from one another regarding their maximum growth temperature. The upper limit for protozoa is around 50°C. Some algae and fungi can grow at temperatures as high as 55 to 60°C. Procaryotes have been found growing at or close to 100°C, the boiling point of water at sea level (*see figure 20.8*). Recently strains growing at even higher temperatures have been discovered (**Box 6.1**). Clearly, procaryotic organisms can grow at much higher temperatures than eucaryotes. It has been suggested that eucaryotes are not able to manufacture organellar membranes that are stable and functional at temperatures above 60°C. The photosynthetic apparatus also appears to be relatively unstable because photosynthetic organisms are not found growing at very high temperatures.

Box 6.1

Life above 100°C

Until recently the highest reported temperature for procaryotic growth was 105°C. It seemed that the upper temperature limit for life was about 100°C, the boiling point of water. Now thermophilic procaryotes have been reported growing in sulfide chimneys or "black smokers," located along rifts and ridges on the ocean floor, that spew sulfide-rich superheated vent water with temperatures above 350°C (see **Box figure**). Evidence has been presented that these microbes can grow and reproduce at 113°C. The pressure present in their habitat is sufficient to keep water liquid (at 265 atm; seawater doesn't boil until 460°C).

The implications of this discovery are many. The proteins, membranes, and nucleic acids of these procaryotes are remarkably temperature stable and provide ideal subjects for studying the ways in which macromolecules and membranes are stabilized. In the future it may be possible to design enzymes that can operate at very high temperatures. Some thermostable enzymes from these organisms have important industrial and scientific uses. For example, the Taq polymerase from the thermophile *Thermus aquaticus* is used extensively in the polymerase chain reaction (*see pp. 326–27*).



Microorganisms such as those in **table 6.5** can be placed in one of five classes based on their temperature ranges for growth (**figure 6.13**).

1. **Psychrophiles** grow well at 0°C and have an optimum growth temperature of 15°C or lower; the maximum is around 20°C. They are readily isolated from Arctic and Antarctic habitats; because 90% of the ocean is 5°C or colder (*see chapter 29*), it constitutes an enormous habitat for psychrophiles. The psychrophilic alga *Chlamydomonas nivalis* can actually turn a snowfield or glacier pink with its bright red spores. Psychrophiles are widespread among bacterial taxa and found in such genera as *Pseudomonas, Vibrio, Alcaligenes, Bacillus, Arthrobacter, Moritella, Photobacterium,* and *Shewanella.* The psychrophilic archaeon *Methanogenium* has recently been isolated from Ace Lake in Antarctica. Psychrophilic microorganisms have adapted to their environment in several ways. Their enzymes, transport systems, and protein synthetic mechanisms function well at low temperatures. The cell membranes of psychrophilic microorganisms have high levels of unsaturated fatty acids and remain semifluid when cold. Indeed, many psychrophiles begin to leak cellular constituents at temperatures higher than 20°C because of cell membrane disruption.
2. Many species can grow at 0 to 7°C even though they have optima between 20 and 30°C, and maxima at about 35°C. These are called **psychrotrophs** or **facultative psychrophiles.** Psychrotrophic bacteria and fungi are major factors in the spoilage of refrigerated foods (*see chapter 41*).
3. **Mesophiles** are microorganisms with growth optima around 20 to 45°C; they often have a temperature minimum of 15 to 20°C. Their maximum is about 45°C or lower. Most microorganisms probably fall within this category. Almost all human pathogens are mesophiles, as might be expected since their environment is a fairly constant 37°C.
4. Some microorganisms are **thermophiles;** they can grow at temperatures of 55°C or higher. Their growth minimum is usually around 45°C and they often have optima between 55 and 65°C. The vast majority are procaryotes although a few algae and fungi are thermophilic (table 6.5). These organisms flourish in many habitats including composts, self-heating hay stacks, hot water lines, and hot springs. Thermophiles differ from mesophiles in having much more heat-stable enzymes and protein synthesis systems able to function at high temperatures. Their membrane lipids are also more saturated than those of mesophiles and have higher melting points; therefore thermophile membranes remain intact at higher temperatures.
5. As mentioned previously, a few thermophiles can grow at 90°C or above and some have maxima above 100°C. Procaryotes that have growth optima between 80°C and about 113°C are called **hyperthermophiles.** They usually do not grow well below 55°C. *Pyrococcus abyssi* and *Pyrodictium occultum* are examples of marine hyperthermophiles found in hot areas of the seafloor.

Table 6.5 Temperature Ranges for Microbial Growth

Microorganism	Cardinal Temperatures (°C)		
	Minimum	Optimum	Maximum
Nonphotosynthetic Procaryotes			
Bacillus psychrophilus	−10	23–24	28–30
Micrococcus cryophilus	−4	10	24
Pseudomonas fluorescens	4	25–30	40
Staphylococcus aureus	6.5	30–37	46
Enterococcus faecalis	0	37	44
Escherichia coli	10	37	45
Neisseria gonorrhoeae	30	35–36	38
Thermoplasma acidophilum	45	59	62
Bacillus stearothermophilus	30	60–65	75
Thermus aquaticus	40	70–72	79
Sulfolobus acidocaldarius	60	80	85
Pyrococcus abyssi	67	96	102
Pyrodictium occultum	82	105	110
Pyrolobus fumarii	90	106	113
Photosynthetic Bacteria			
Rhodospirillum rubrum	ND[a]	30–35	ND
Anabaena variabilis	ND	35	ND
Oscillatoria tenuis	ND	ND	45–47
Synechococcus eximius	70	79	84
Eucaryotic Algae			
Chlamydomonas nivalis	–36	0	4
Fragilaria sublinearis	–2	5–6	8–9
Chlorella pyrenoidosa	ND	25–26	29
Euglena gracilis	ND	23	ND
Skeletonema costatum	6	16–26	>28
Cyanidium caldarium	30–34	45–50	56
Fungi			
Candida scottii	0	4–15	15
Saccharomyces cerevisiae	1–3	28	40
Mucor pusillus	21–23	45–50	50–58
Protozoa			
Amoeba proteus	4–6	22	35
Naegleria fowleri	20–25	35	40
Trichomonas vaginalis	25	32–39	42
Paramecium caudatum		25	28–30
Tetrahymena pyriformis	6–7	20–25	33
Cyclidium citrullus	18	43	47

[a]ND, no data.

1. Define pH, acidophile, neutrophile, and alkalophile. How can microorganisms change the pH of their environment, and how does the microbiologist minimize this effect?
2. What are cardinal temperatures?
3. Why does the growth rate rise with increasing temperature and then fall again at higher temperatures?
4. Define psychrophile, psychrotroph, mesophile, thermophile, and hyperthermophile.

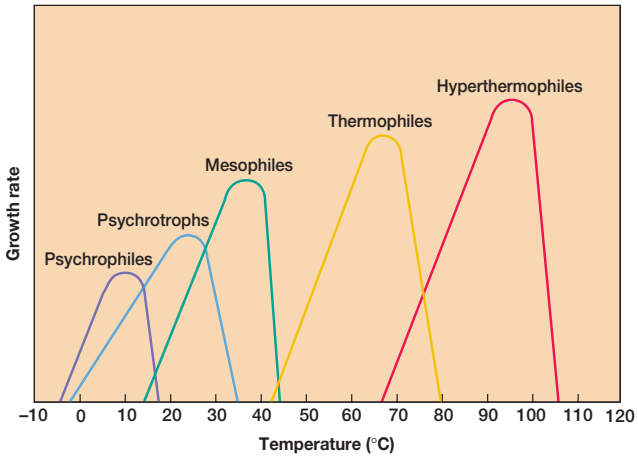


Figure 6.13 Temperature Ranges for Microbial Growth. Microorganisms can be placed in different classes based on their temperature ranges for growth. They are ranked in order of increasing growth temperature range as psychrophiles, psychrotrophs, mesophiles, thermophiles, and hyperthermophiles. Representative ranges and optima for these five types are illustrated here.

Oxygen Concentration

An organism able to grow in the presence of atmospheric O_2 is an **aerobe,** whereas one that can grow in its absence is an **anaerobe.** Almost all multicellular organisms are completely dependent on atmospheric O_2 for growth—that is, they are **obligate aerobes** (table 6.3). Oxygen serves as the terminal electron acceptor for the electron-transport chain in aerobic respiration. In addition, aerobic eucaryotes employ O_2 in the synthesis of sterols and unsaturated fatty acids. **Facultative anaerobes** do not require O_2 for growth but do grow better in its presence. In the presence of oxygen they will use aerobic respiration. **Aerotolerant anaerobes** such as *Enterococcus faecalis* simply ignore O_2 and grow equally well whether it is present or not. In contrast, **strict** or **obligate anaerobes** (e.g., *Bacteroides, Fusobacterium, Clostridium pasteurianum, Methanococcus*) do not tolerate O_2 at all and die in its presence. Aerotolerant and strict anaerobes cannot generate energy through respiration and must employ fermentation or anaerobic respiration pathways for this purpose. Finally, there are aerobes such as *Campylobacter,* called **microaerophiles,** that are damaged by the normal atmospheric level of O_2 (20%) and require O_2 levels below the range of 2 to 10% for growth. The nature of bacterial O_2 responses can be readily determined by growing the bacteria in culture tubes filled with a solid culture medium or a special medium like thioglycollate broth, which contains a reducing agent to lower O_2 levels (**figure 6.14**). Electron transport and aerobic respiration (pp. 184–89); Fermentation (pp. 179–81); Anaerobic respiration (pp. 190–91)

A microbial group may show more than one type of relationship to O_2. All five types are found among the procaryotes and protozoa. Fungi are normally aerobic, but a number of species—particularly among the yeasts—are facultative anaerobes. Algae

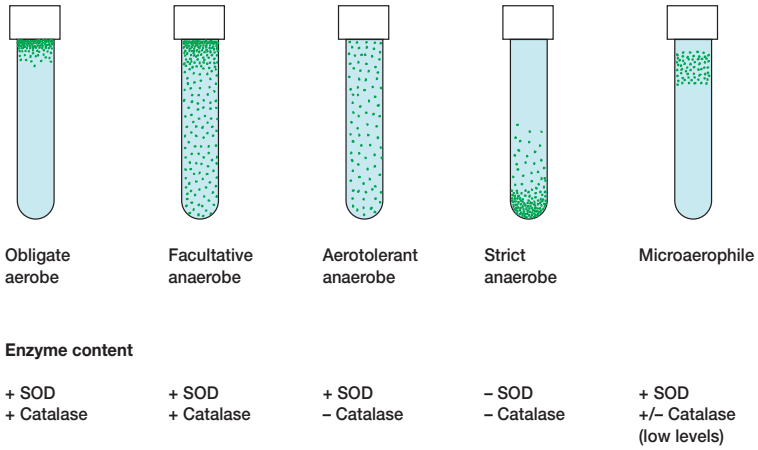


Figure 6.14 Oxygen and Bacterial Growth. An illustration of the growth of bacteria with varying responses to oxygen. Each dot represents an individual bacterial colony within the agar or on its surface. The surface, which is directly exposed to atmospheric oxygen, will be aerobic. The oxygen content of the medium decreases with depth until the medium becomes anaerobic toward the bottom of the tube. The presence and absence of the enzymes superoxide dismutase (SOD) and catalase for each type are shown.

are almost always obligate aerobes. It should be noted that the ability to grow in both aerobic and anaerobic environments provides considerable flexibility and is an ecological advantage.

Although strict anaerobes are killed by O_2, they may be recovered from habitats that appear to be aerobic. In such cases they associate with facultative anaerobes that use up the available O_2 and thus make the growth of strict anaerobes possible. For example, the strict anaerobe *Bacteroides gingivalis* lives in the mouth where it grows in the anaerobic crevices around the teeth.

These different relationships with O_2 appear due to several factors, including the inactivation of proteins and the effect of toxic O_2 derivatives. Enzymes can be inactivated when sensitive groups like sulfhydryls are oxidized. A notable example is the nitrogen-fixation enzyme nitrogenase (*see section 10.4*), which is very oxygen sensitive.

Oxygen accepts electrons and is readily reduced because its two outer orbital electrons are unpaired. Flavoproteins (*see section 8.5*), several other cell constituents, and radiation (pp. 130–31) promote oxygen reduction. The result is usually some combination of the reduction products **superoxide radical, hydrogen peroxide,** and **hydroxyl radical.**

$$O_2 + e^- \rightarrow O_2^{-}\cdot \text{ (superoxide radical)}$$

$$O_2^{-}\cdot + e^- + 2H^+ \longrightarrow H_2O_2 \text{ (hydrogen peroxide)}$$

$$H_2O_2 + e^- + H^+ \longrightarrow H_2O + OH\cdot \text{ (hydroxyl radical)}$$

These products of oxygen reduction are extremely toxic because they are powerful oxidizing agents and rapidly destroy cellular constituents. A microorganism must be able to protect itself against such oxygen products or it will be killed. Neutrophils and macrophages use these toxic oxygen products to destroy invading pathogens. Oxygen-dependent killing of pathogens (pp. 718–20)

Many microorganisms possess enzymes that afford protection against toxic O_2 products. Obligate aerobes and facultative anaerobes usually contain the enzymes **superoxide dismutase (SOD)** and **catalase,** which catalyze the destruction of superoxide radical and hydrogen peroxide, respectively. Peroxidase also can be used to destroy hydrogen peroxide.

$$2O_2^{-}\cdot + 2H^+ \xrightarrow{\text{superoxide dismutase}} O_2 + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

$$H_2O_2 + NADH + H^+ \xrightarrow{\text{peroxidase}} 2H_2O + NAD^+$$

Aerotolerant microorganisms may lack catalase but almost always have superoxide dismutase. The aerotolerant *Lactobacillus plantarum* uses manganous ions instead of superoxide dismutase to destroy the superoxide radical. All strict anaerobes lack both enzymes or have them in very low concentrations and therefore cannot tolerate O_2.

Because aerobes need O_2 and anaerobes are killed by it, radically different approaches must be used when growing the two types of microorganisms. When large volumes of aerobic microorganisms are cultured, either the culture vessel is shaken to aerate the medium or sterile air must be pumped through the culture vessel. Precisely the opposite problem arises with anaerobes; all O_2 must be excluded. This can be accomplished in several ways. (1) Special anaerobic media containing reducing agents such as thioglycollate or cysteine may be used. The medium is boiled during preparation to dissolve its components; boiling also drives off oxygen very effectively. The reducing agents will eliminate any dissolved O_2 remaining within the medium so that anaerobes can grow beneath its surface. (2) Oxygen also may be eliminated from an anaerobic system by removing air with a vacuum pump and flushing out residual O_2 with nitrogen gas (**figure 6.15**). Often CO_2 as well as nitrogen is added to the chamber since many anaerobes require a small amount of CO_2 for best growth. (3) One of the most popular ways of culturing small numbers of anaerobes is by use of a GasPak jar (**figure 6.16**). In this procedure the environment is made anaerobic by using hydrogen and a palladium catalyst to remove

O_2 through the formation of water. The reducing agents in anaerobic agar also remove oxygen, as mentioned previously. (4) Plastic bags or pouches make convenient containers when only a few samples are to be incubated anaerobically. These have a catalyst and calcium carbonate to produce an anaerobic, carbon-dioxide-rich atmosphere. A special solution is added to the pouch's reagent compartment; petri dishes or other containers are placed in the pouch; it then is clamped shut and placed in an incubator. A laboratory may make use of all these techniques since each is best suited for different purposes.

Figure 6.15 An Anaerobic Work Chamber and Incubator. This anaerobic system contains an oxygen-free work area and an incubator. The interchange compartment on the right of the work area allows materials to be transferred inside without exposing the interior to oxygen. The anaerobic atmosphere is maintained largely with a vacuum pump and nitrogen purges. The remaining oxygen is removed by a palladium catalyst and hydrogen. The oxygen reacts with hydrogen to form water, which is absorbed by desiccant.

1. Describe the five types of O_2 relationships seen in microorganisms.
2. For what do aerobes use O_2? Why is O_2 toxic to many microorganisms and how do they protect themselves?
3. Describe four ways in which anaerobes may be cultured.

Pressure

Most organisms spend their lives on land or on the surface of water, always subjected to a pressure of 1 atmosphere (atm), and are never affected significantly by pressure. Yet the deep sea (ocean of 1,000 m or more in depth) is 75% of the total ocean volume. The hydrostatic pressure can reach 600 to 1,100 atm in the deep sea, while the temperature is about 2 to 3°C. Despite these extremes, bacteria survive and adapt. Many are **barotolerant:** increased pressure does adversely affect them but not as much as it does nontolerant bacteria. Some bacteria in the gut of deep-sea invertebrates such as amphipods and holothurians are truly **barophilic**—they grow more rapidly at high pressures. These gut bacteria may play an important role in nutrient recycling in the deep sea. One barophile has been recovered from the Mariana trench near the Philippines (depth about 10,500 m) that is actually unable to grow at pressures below about 400 to 500 atm when incubated at 2°C. Thus far, barophiles have been found among several bacterial genera (e.g., *Photobacterium, Shewanella, Colwellia*). Some members of the Archaea are thermobarophiles (e.g., *Pyrococcus* spp., *Methanococcus jannaschii*). The marine environment (pp. 644–48)

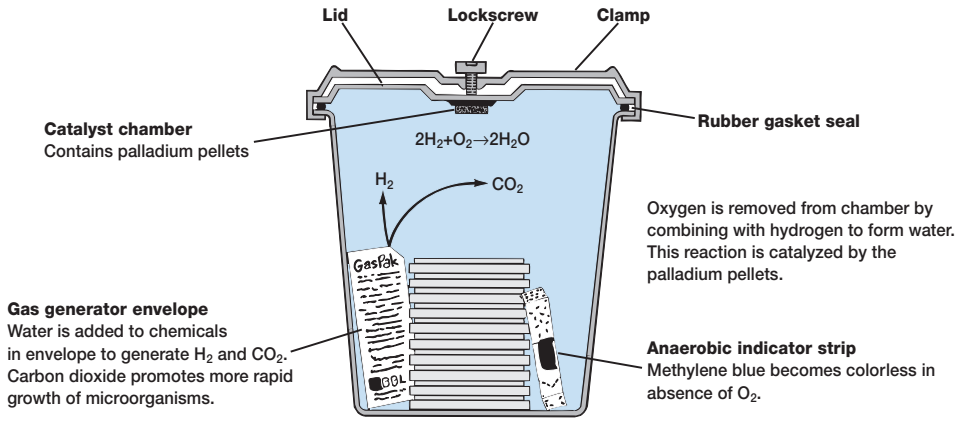


Figure 6.16 The GasPak Anaerobic System. Hydrogen and carbon dioxide are generated by a GasPak envelope. The palladium catalyst in the chamber lid catalyzes the formation of water from hydrogen and oxygen, thereby removing oxygen from the sealed chamber.

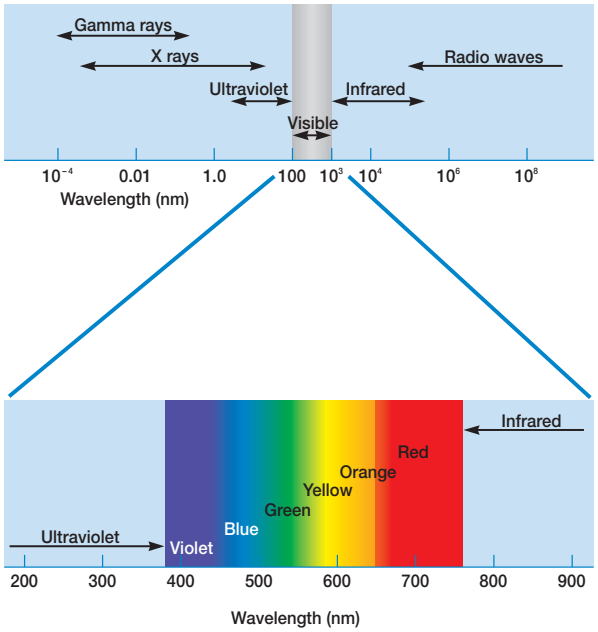


Figure 6.17 **The Electromagnetic Spectrum.** The visible portion of the spectrum is expanded at the bottom of the figure.

Radiation

Our world is bombarded with electromagnetic radiation of various types (**figure 6.17**). This radiation often behaves as if it were composed of waves moving through space like waves traveling on the surface of water. The distance between two wave crests or troughs is the wavelength. 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. Electromagnetic radiation also acts like a stream of energy packets called photons, each photon having a quantum of energy whose value will depend on the wavelength of the radiation.

Sunlight is the major source of radiation on the Earth. It includes visible light, ultraviolet (UV) radiation, infrared rays, and radio waves. Visible light is a most conspicuous and important aspect of our environment: all life is dependent on the ability of photosynthetic organisms to trap the light energy of the sun. Almost 60% of the sun's radiation is in the infrared region rather than the visible portion of the spectrum. Infrared is the major source of the Earth's heat. At sea level, one finds very little ultraviolet radiation below about 290 to 300 nm. UV radiation of wavelengths shorter than 287 nm is absorbed by O_2 in the Earth's atmosphere; this process forms a layer of ozone between 25 and 30 miles above the Earth's surface. The ozone layer then absorbs somewhat longer UV rays and reforms O_2. This elimination of UV radiation is crucial because it is quite damaging to living systems (*see chapter 11*). The fairly even distribution of sunlight throughout the visible spectrum accounts for the fact that sunlight is generally "white." Microbial photosynthesis (pp. 195–201)

Many forms of electromagnetic radiation are very harmful to microorganisms. This is particularly true of **ionizing radiation,** radiation of very short wavelength or high energy, which can cause atoms to lose electrons or ionize. Two major forms of ionizing radiation are (1) X rays, which are artificially produced, and (2) gamma rays, which are emitted during radioisotope decay. Low levels of ionizing radiation will produce mutations and may indirectly result in death, whereas higher levels are directly lethal. Although microorganisms are more resistant to ionizing radiation than larger organisms, they will still be destroyed by a sufficiently large dose. Ionizing radiation can be used to sterilize items. Some procaryotes (e.g., *Deinococcus radiodurans*) and bacterial endospores can survive large doses of ionizing radiation. Use of radiation in destroying microorganisms (p. 144); *Deinococcus* (p. 468)

A variety of changes in cells are due to ionizing radiation; it breaks hydrogen bonds, oxidizes double bonds, destroys ring structures, and polymerizes some molecules. Oxygen enhances these destructive effects, probably through the generation of hydroxyl radicals (OH·). Although many types of constituents can be affected, it is reasonable to suppose that destruction of DNA is the most important cause of death.

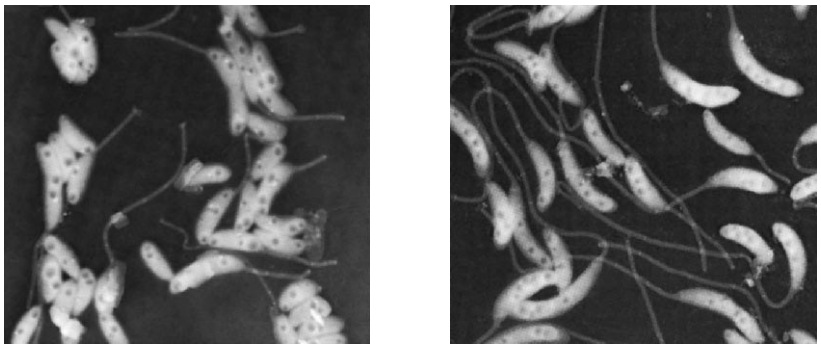
Ultraviolet (UV) radiation, mentioned earlier, kills all kinds of microorganisms due to its short wavelength (approximately from 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. The primary mechanism of UV damage is the formation of thymine dimers in DNA (*see pp. 248–49*). Two adjacent thymines in a DNA strand are covalently joined to inhibit DNA replication and function. This damage is repaired in several ways. In **photoreactivation,** blue light is used by a photoreactivating enzyme to split thymine dimers. A short sequence containing the thymine dimer can also be excised and replaced. This process occurs in the absence of light and is called **dark reactivation.** Damage also can be repaired by the recA protein in recombination repair and SOS repair. When UV exposure is too heavy, the damage is so extensive that repair is impossible. DNA repair mechanisms (pp. 254–56)

Although very little UV radiation below 290 to 300 nm reaches the earth's surface, near-UV radiation between 325 and 400 nm can harm microorganisms. Exposure to near-UV radiation induces tryptophan breakdown to toxic photoproducts. It appears that these toxic tryptophan photoproducts plus the near-UV radiation itself produce breaks in DNA strands. The precise mechanism is not known, although it is different from that seen with 260 nm UV.

Visible light is immensely beneficial because it is the source of energy for photosynthesis. Yet even visible light, when present in sufficient intensity, can damage or kill microbial cells. Usually pigments called photosensitizers and O_2 are required. All microorganisms possess pigments like chlorophyll, bacteriochlorophyll, cytochromes, and flavins, which can absorb light energy, become excited or activated, and act as photosensitizers. The excited photosensitizer (P) transfers its energy to O_2 generating **singlet oxygen** ($^1O_2$).

$$P \xrightarrow{\text{light}} P \text{ (activated)}$$

$$P \text{ (activated)} + O_2 \longrightarrow P + {}^1O_2$$



(a) (b)

Figure 6.18 Morphology and Nutrient Absorption. Microorganisms can change their morphology in response to starvation and different limiting factors to improve their ability to survive. (**a**) *Caulobacter* has relatively short stalks when nitrogen is limiting. (**b**) The stalks are extremely long under phosphorus-limited conditions.

Singlet oxygen is a very reactive, powerful oxidizing agent that will quickly destroy a cell. It is probably the major agent employed by phagocytes to destroy engulfed bacteria (*see section 31.8*).

Many microorganisms that are airborne or live on exposed surfaces use carotenoid pigments for protection against photooxidation. Carotenoids effectively quench singlet oxygen—that is, they absorb energy from singlet oxygen and convert it back into the unexcited ground state. Both photosynthetic and nonphotosynthetic microorganisms employ pigments in this way.

1. What are barotolerant and barophilic bacteria? Where would you expect to find them?
2. List the types of electromagnetic radiation in the order of decreasing energy or increasing wavelength.
3. Why is it so important that the Earth receives an adequate supply of sunlight? What is the importance of ozone formation?
4. How do ionizing radiation, ultraviolet radiation, and visible light harm microorganisms? How do microorganisms protect themselves against damage from UV and visible light?

6.5 Microbial Growth in Natural Environments

The previous section surveyed the effects on microbial growth of individual environmental factors such as water availability, pH, and temperature. Although microbial ecology will be introduced in more detail at a later point, we will now briefly consider the effect of the environment as a whole on microbial growth. Microbial ecology (chapters 28–30)

Growth Limitation by Environmental Factors

The microbial environment is complex and constantly changing. Characteristically microorganisms in a particular location are exposed to many overlapping gradients of nutrients and various other environmental factors. This is particularly true of microorganisms growing in biofilms. Microorganisms will grow in "microenvironments" until an environmental or nutritional factor limits growth. **Liebig's law of the minimum** states that the total biomass of an organism will be determined by the nutrient present in the lowest concentration relative to the organism's requirements. This law applies in both the laboratory (figure 6.2) and in terrestrial and aquatic environments. An increase in a limiting essential nutrient such as phosphate will result in an increase in the microbial population until some other nutrient becomes limiting. If a specific nutrient is limiting, changes in other nutrients will have no effect. The situation may be even more complex than this. Multiple limiting factors can influence a population over time. Furthermore, as we have seen, factors such as temperature, pH, light, and salinity influence microbial populations and limit growth. **Shelford's law of tolerance** states that there are limits to environmental factors below and above which a microorganism cannot survive and grow, regardless of the nutrient supply. This can readily be seen for temperature in figure 6.13. Each microorganism has a specific temperature range in which it can grow. The same rule applies to other factors such as pH, oxygen level, and hydrostatic pressure in the marine environment. The growth of a microorganism depends on both the nutrient supply and its tolerance of the environmental conditions. Biofilms (pp. 620–22)

Most microorganisms are confronted with deficiencies that limit their activities except when excess nutrients allow unlimited growth. Such rapid growth will quickly deplete nutrients and possibly result in the release of toxic waste products, which will limit further growth.

In response to low nutrient levels (**oligotrophic environments**) and intense competition, many microorganisms become more competitive in nutrient capture and exploitation of available resources. Often the organism's morphology will change in order to increase its surface area and ability to absorb nutrients. This can involve conversion of rod-shaped procaryotes to "mini" and "ultramicro" cells or changes in the morphology of prosthecate (*see pp. 490–92*) procaryotes (**figure 6.18**), in response to starvation. Nutrient deprivation induces many other changes as discussed previously. For example, microorganisms can undergo a step-by-step shutdown of metabolism except for housekeeping maintenance genes.

Many factors can alter nutrient levels in oligotrophic environments. Microorganisms may sequester critical limiting nutrients, such as iron, making them less available to competitors. The atmosphere can contribute essential nutrients and support

microbial growth. This is seen in the laboratory as well as natural environments. Airborne organic substances have been found to stimulate microbial growth in dilute media, and enrichment of growth media by airborne organic matter can allow significant populations of microorganisms to develop. Even distilled water, which already contains traces of organic matter, can absorb one-carbon compounds from the atmosphere and grow microorganisms. The presence of such airborne nutrients and microbial growth, if not detected, can affect experiments in biochemistry and molecular biology, as well as studies of microorganisms growing in oligotrophic environments.

Natural substances also can directly inhibit microbial growth and reproduction in low-nutrient environments. These agents include phenolics, tannins, ammonia, ethylene, and volatile sulfur compounds. This may be a means by which microorganisms avoid expending limited energy reserves until an adequate supply of nutrients becomes available. Such chemicals are also important in plant pathology and may aid in controlling soil-borne microbial diseases.

Counting Viable But Nonculturable Vegetative Procaryotes

In order to study the growth of natural procaryotic populations outside the laboratory, it is essential to determine the number of viable microorganisms present. For most of microbiology's history, a viable microorganism has been defined as one that is able to grow actively, resulting in the formation of a colony or visible turbidity in a liquid medium. John R. Postgate of the University of Sussex in England was one of the first to note that microorganisms stressed by survival in natural habitats—or in many selective laboratory media—were particularly sensitive to secondary stresses. Such stresses can produce viable microorganisms without the ability to grow on media normally used for their cultivation. To determine the growth potential of such microorganisms, Postgate developed what is now called the Postgate Microviability Assay, in which microorganisms are cultured in a thin agar film under a coverslip. The ability of a cell to change its morphology, even if it does not grow beyond the single-cell stage, indicates that the microorganism does show "life signs."

Since that time many workers have developed additional sensitive microscopic and isotopic procedures to evaluate the presence and significance of these viable but nonculturable bacteria in both lab and field. For example, levels of fluorescent antibody and acridine orange–stained cells often are compared with population counts obtained by the most probable number (MPN) method (*see pp. 654–55*) and plate counts using selective and nonselective media. The release of radioactive-labeled cell materials also is used to monitor stress effects on microorganisms. Despite these advances the estimation of substrate-responsive viable cells by Postgate's method is still important. These studies show that even when bacteria such as *Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae, Enterobacter aerogenes,* and *Enterococcus faecalis* have lost their ability to grow on conventional laboratory media using standard cultural techniques, they still might be able to play a role in infectious disease.

The situation in natural environments with mixed populations is much more complex. Here, where often only 1 to 10% of observable cells are able to form colonies, the microbiologist is attempting to grow microorganisms that perhaps never have been cultured or characterized. In the future it is possible that media or proper environmental conditions for their growth will be developed. At present, molecular techniques involving PCR amplification and small subunit ribosomal RNA analysis are increasingly used to analyze the diversity of uncultured microbial populations (*see pp. 626–29*).

Quorum Sensing and Microbial Populations

For decades microbiologists tended to think of bacterial populations as collections of individuals growing and behaving independently. More recently it has become clear that many bacteria can communicate with one another and behave cooperatively. A major way in which this cooperation is accomplished is by a process known as **quorum sensing** or autoinduction. This is a phenomenon in which bacteria monitor their own population density through sensing the levels of signal molecules, sometimes called autoinducers because they can stimulate the cell that releases them. The concentration of these signal molecules increases along with the bacterial population until it rises to a specific threshold and signals the bacteria that the population density has reached a critical level or quorum. The bacteria then begin expressing sets of quorum-dependent genes. Quorum sensing has been found among both gram-negative and gram-positive bacteria.

Quorum sensing makes good practical sense. Take the production and release of extracellular enzymes as an example. If such enzymes were released by only a few bacteria, they would diffuse away and be rendered ineffective because of dilution. With control by quorum sensing, the bacteria reach a high population density before they release enzymes, and as a consequence enzyme levels are concentrated enough to have significant effects. This is an advantage within a host's body as well as in the soil or an aquatic habitat. If a pathogen can reach high levels at a particular site before producing virulence factors and escaping into surrounding host tissues, it has a much better chance of counteracting host defenses and successfully spreading throughout the host's body. This explains another pattern in quorum sensing. It seems to be very important in many bacteria that establish symbiotic or parasitic relationships with hosts.

Quorum sensing was first discovered in gram-negative bacteria and is best understood in these microorganisms. The most common signals in gram-negative bacteria are acyl *h*omo*s*erine *l*actones (HSLs). These are small molecules composed of a 4- to 14-carbon acyl chain attached by an amide bond to homoserine lactone (**figure 6.19*a***). The acyl chain may have a keto group or hydroxyl group on its third carbon. Acyl HSLs diffuse into the target cell (figure 6.19*b*). Once they reach a sufficiently high level, acyl HSLs bind to special receptor proteins and trigger a conformational change. Usually the activated complexes act as inducers—that is, they bind to target sites on the DNA and stimulate transcription of quorum-sensitive genes. The gene needed to synthesize acyl HSL is also produced frequently, thus amplifying

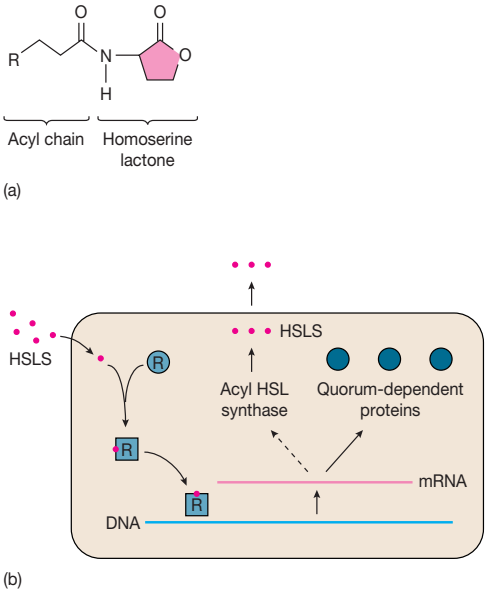


Figure 6.19 Quorum Sensing in Gram-Negative Bacteria. (**a**) A generalized structure for acyl homoserine lactone, the best-known quorum sensing signal or autoinducer. (**b**) A schematic diagram giving an overview of the way in which quorum sensing functions in many gram-negative bacteria. The receptor protein that acts as an inducer is labeled R. The dashed lines indicate that acyl HSL synthase is not always made in response to the autoinducer. See text for more details.

the effect by the production and release of more autoinducer molecules. Induction and repression of genetic activity (pp. 275–78)

Many different processes are sensitive to acyl HSL signals and quorum sensing in gram-negative bacteria. Some well-studied examples are (1) bioluminescence production by *Vibrio fischeri,* (2) *Pseudomonas aeruginosa* synthesis and release of virulence factors, (3) conjugal transfer of genetic material by *Agrobacterium tumefaciens,* and (4) antibiotic production by *Erwinia carotovora* and *Pseudomonas aureofaciens.*

Gram-positive bacteria also regulate activities by quorum sensing, often using an oligopeptide signal. Good examples are mating in *Enterococcus faecalis,* competence induction in *Streptococcus pneumoniae,* stimulation of sporulation by *Bacillus subtilis,* and production of many toxins and other virulence factors by *Staphylococcus aureus.* Quorum sensing even stimulates the development of aerial mycelia and the production of streptomycin by *Streptomyces griseus.* In this case, the signal seems to be γ-butyrolactone rather than an oligopeptide.

An interesting and important function of quorum sensing is to promote the formation of mature biofilms by the pathogen *Pseudomonas aeruginosa,* and it may play a role in cystic fibrosis. Biofilm formation makes sense for the pathogen because biofilms protect against antibiotics and detergents. Quorum sensing should be very effective within biofilms because there will be less dilution and acyl HSL levels will increase rapidly. Under such circumstances, two different bacteria might stimulate each other by releasing similar signals; this appears to be the case in biofilms containing the pathogens *P. aeruginosa* and *Burkholderia cepacia.*

Quorum sensing is an example of what might be called multicellular behavior in that many individual cells communicate and coordinate their activities to act as a unit. Other examples of such complex behavior is pattern formation in colonies (*see pp. 108–10*) and fruiting body formation in the myxobacteria (*see pp. 512–13*).

1. How are Liebig's law of the minimum and Shelford's law of tolerance related?
2. Describe how microorganisms respond to oligotrophic environments.
3. Briefly discuss the Postgate microviability assay and other ways in which viable but nonculturable microorganisms can be counted or studied.
4. What is quorum sensing? Describe how it occurs and briefly discuss its importance to microorganisms.

Summary

1. Growth is an increase in cellular constituents and results in an increase in cell size, cell number, or both.
2. When microorganisms are grown in a closed system or batch culture, the resulting growth curve usually has four phases: the lag, exponential or log, stationary, and death phases (**figure 6.1**).
3. In the exponential phase, the population number doubles at a constant interval called the doubling or generation time (**figure 6.3**). The mean growth rate constant (k) is the reciprocal of the generation time.
4. Exponential growth is balanced growth, cell components are synthesized at constant rates relative to one another. Changes in culture conditions (e.g., in shift-up and shift-down experiments) lead to unbalanced growth. A portion of the available nutrients is used to supply maintenance energy.
5. Microbial populations can be counted directly with counting chambers, electronic counters, or fluorescence microscopy. Viable counting techniques such as the spread plate, the pour plate, or the membrane filter can be employed.
6. Population changes also can be followed by determining variations in microbial mass through the measurement of dry weight, turbidity, or the amount of a cell component.
7. Microorganisms can be grown in an open system in which nutrients are constantly provided and wastes removed.
8. A continuous culture system is an open system that can maintain a microbial population in the log phase. There are two types of these systems: chemostats and turbidostats.
9. Most bacteria, algae, and fungi have rigid cell walls and are hypertonic to the habitat because of solutes such as amino acids, polyols, and potassium ions. The amount of water actually available to microorganisms is expressed in terms of the water activity (a_w).
10. Although most microorganisms will not grow well at water activities below 0.98 due to plasmolysis and associated effects, osmotolerant organisms survive and even flourish at low a_w values. Halophiles actually require high sodium chloride concentrations for growth (**table 6.3**).

11. Each species of microorganism has an optimum pH for growth and can be classified as an acidophile, neutrophile, or alkalophile.
12. Microorganisms can alter the pH of their surroundings, and most culture media must be buffered to stabilize the pH.
13. Microorganisms have distinct temperature ranges for growth with minima, maxima, and optima—the cardinal temperatures. These ranges are determined by the effects of temperature on the rates of catalysis, protein denaturation, and membrane disruption.
14. There are five major classes of microorganisms with respect to temperature preferences: (1) psychrophiles, (2) facultative psychrophiles or psychrotrophs, (3) mesophiles, (4) thermophiles and (5) hyperthermophiles (**figure 6.13 and table 6.3**).
15. Microorganisms can be placed into at least five different categories based on their response to the presence of O_2: obligate aerobes, facultative anaerobes, aerotolerant anaerobes, strict or obligate anaerobes, and microaerophiles (**figure 6.14 and table 6.3**).
16. Oxygen can become toxic because of the production of hydrogen peroxide, superoxide radical, and hydroxyl radical. These are destroyed by the enzymes superoxide dismutase, catalase, and peroxidase.
17. Most deep-sea microorganisms are barotolerant, but some are barophilic and require high pressure for optimal growth.
18. High-energy or short-wavelength radiation harms organisms in several ways. Ionizing radiation—X rays and gamma rays—ionizes molecules and destroys DNA and other cell components.
19. Ultraviolet (UV) radiation induces the formation of thymine dimers and strand breaks in DNA. Such damage can be repaired by photoreactivation or dark reactivation mechanisms.
20. Visible light can provide energy for the formation of reactive singlet oxygen, which will destroy cells.
21. Microbial growth in natural environments is profoundly affected by nutrient limitations and other adverse factors. Some microorganisms can be viable but unculturable and must be studied with special techniques.
22. Often, bacteria will communicate with one another in a density-dependent way and carry out a particular activity only when a certain population density is reached. This phenomenon is called quorum sensing.

Key Terms

acidophile *123*
aerobe *127*
aerotolerant anaerobe *127*
alkalophile *123*
anaerobe *127*
balanced growth *114*
barophilic *129*
barotolerant *129*
batch culture *113*
cardinal temperatures *125*
catalase *128*
chemostat *120*
coenocytic *113*
colony forming units (CFU) *118*
compatible solutes *121*
continuous culture system *120*
dark reactivation *130*
death phase *115*
doubling time *115*
exponential phase *114*
extremophiles *121*
facultative anaerobe *127*
facultative psychrophiles *126*
generation time *115*
growth *113*
halophile *123*
hydrogen peroxide *128*
hydroxyl radical *128*
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ionizing radiation *130*
lag phase *113*
Liebig's law of the minimum *131*
log phase *114*
maintenance energy *121*
mean generation time *116*
mean growth rate constant (*k*) *116*
membrane filter *118*
mesophile *126*
microaerophile *127*
neutrophile *123*
obligate aerobe *127*
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oligotrophic environment *131*
osmotolerant *122*
photoreactivation *130*
psychrophile *126*
psychrotroph *126*
quorum sensing *132*
Shelford's law of tolerance *131*
singlet oxygen *130*
starvation proteins *115*
stationary phase *114*
strict anaerobe *127*
superoxide dismutase (SOD) *128*
superoxide radical *128*
thermophile *126*
turbidostat *121*
ultraviolet (UV) radiation *130*
unbalanced growth *114*
water activity (a_w) *122*

Questions for Thought and Review

1. Discuss the reasons why a culture might have a long lag phase after inoculation.
2. Why can't one always tell when a culture enters the death phase by the use of total cell counts?
3. Calculate the mean growth rate and generation time of a culture that increases in the exponential phase from 5×10^2 to 1×10^8 cells in 12 hours.
4. If the generation time is 90 minutes and the initial population contains 10^3 cells, how many bacteria will there be after 8 hours of exponential growth?
5. Why are continuous culture systems so useful to microbiologists?
6. How do bacterial populations respond in shift-up and shift-down experiments? Account for their behavior in molecular terms.
7. Does the internal pH remain constant despite changes in the external pH? How might this be achieved? Explain how extreme pH values might harm microorganisms.
8. What metabolic and structural adaptations for extreme temperatures have psychrophiles and thermophiles made?
9. Why are generation times in nature usually much longer than in culture?

Critical Thinking Questions

1. As an alternative to diffusable signals, suggest another mechanism by which bacteria can quorum sense.
2. Design an "enrichment" culture medium and a protocol for the isolation and purification of a soil bacterium (e.g., *Bacillus subtilis*) from a sample of soil. Note possible contaminants and competitors. How will you adjust conditions of growth and what conditions will be adjusted to differentially enhance the growth of the *Bacillus?*

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