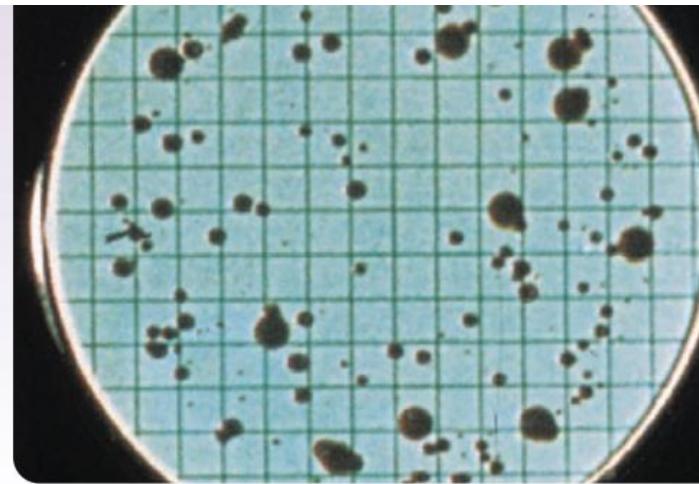


# 6

## 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.

### PREVIEW

- Most prokaryotes reproduce by binary fission. Although simpler than mitosis and meiosis, binary fission and the prokaryotic cell cycle are still poorly understood.
- Growth is defined as an increase in cellular constituents and may result in an increase in a microorganism's size, population number, or both.
- 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.
- 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.
- Water availability, pH, temperature, oxygen concentration, pressure, radiation, and a number of other environmental factors influence microbial growth. Yet many microorganisms, and particularly prokaryotes, have managed to adapt and flourish under environmental extremes that would destroy most higher organisms.
- In the natural environment, growth is often severely limited by available nutrient supplies and many other environmental factors.
- Many microorganisms form biofilms in natural environments. This is an important survival strategy.
- Microbes can communicate with each other and behave cooperatively using population density-dependent signals.

In chapter 5 we emphasize 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. Eukaryotic cells also employ endocytosis.

Chapter 6 concentrates more directly on prokaryotic reproduction and growth. First we describe binary fission, the type of cell division most frequently observed among prokaryotes, and the prokaryotic cell cycle. Cell reproduction leads to an increase in population size, so we consider growth and the ways in which it can be measured next. Then we discuss continuous culture techniques. An account of the influence of environmental factors on microbial growth and microbial growth in natural environments completes the chapter.

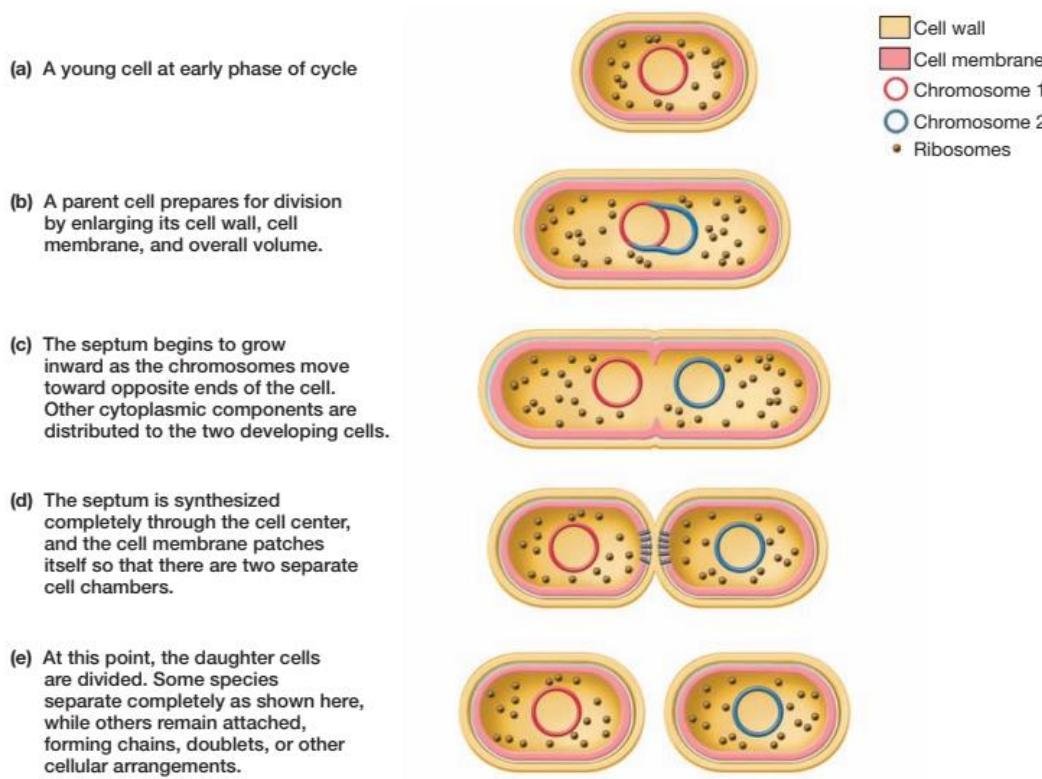
**Growth** may be defined as an increase in cellular constituents. It leads to a rise in cell number when microorganisms reproduce by processes like budding or binary fission. Growth also results when cells simply become longer or larger. If the microorganism is **coenocytic**—that is, a multinucleate 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.

### 6.1 THE PROKARYOTIC CELL CYCLE

The **cell cycle** is the complete sequence of events extending from the formation of a new cell through the next division. Most prokaryotes reproduce by **binary fission**, although some prokaryotes

*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



**Figure 6.1** Binary Fission.

reproduce by budding, fragmentation, and other means (**figure 6.1**). Binary fission is a relatively simple type of cell division: the cell elongates, replicates its chromosome, and separates the newly formed DNA molecules so there is one chromosome in each half of the cell. Finally, a septum (or cross wall) is formed at midcell, dividing the parent cell into two progeny cells, each having its own chromosome and a complement of other cellular constituents.

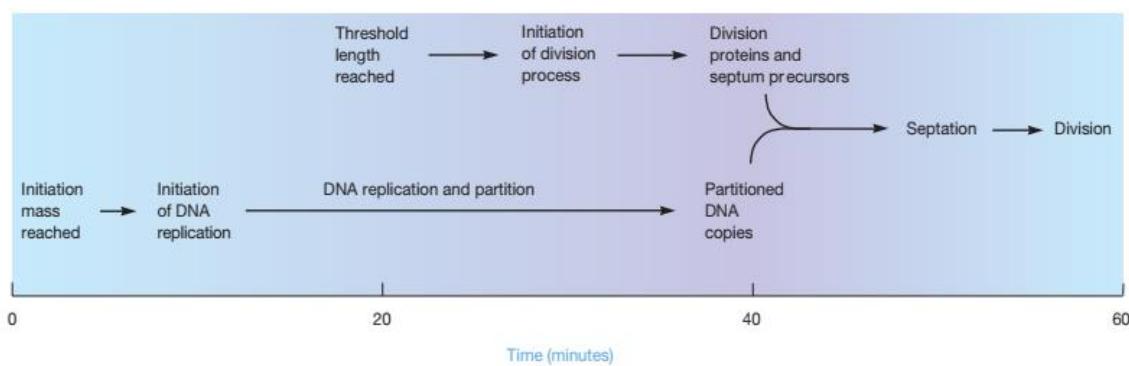
Despite the apparent simplicity of the prokaryotic cell cycle, it is poorly understood. The cell cycles of *Escherichia coli*, *Bacillus subtilis*, and the aquatic microbe *Caulobacter crescentus* have been examined extensively, and our understanding of the cell cycle is based largely on these studies. Two pathways function during the cell cycle (**figure 6.2**): one pathway replicates and partitions the DNA into the progeny cells, the other carries out cytokinesis (septum formation and formation of progeny cells). Although these pathways overlap, it is easiest to consider them separately.

#### Chromosome Replication and Partitioning

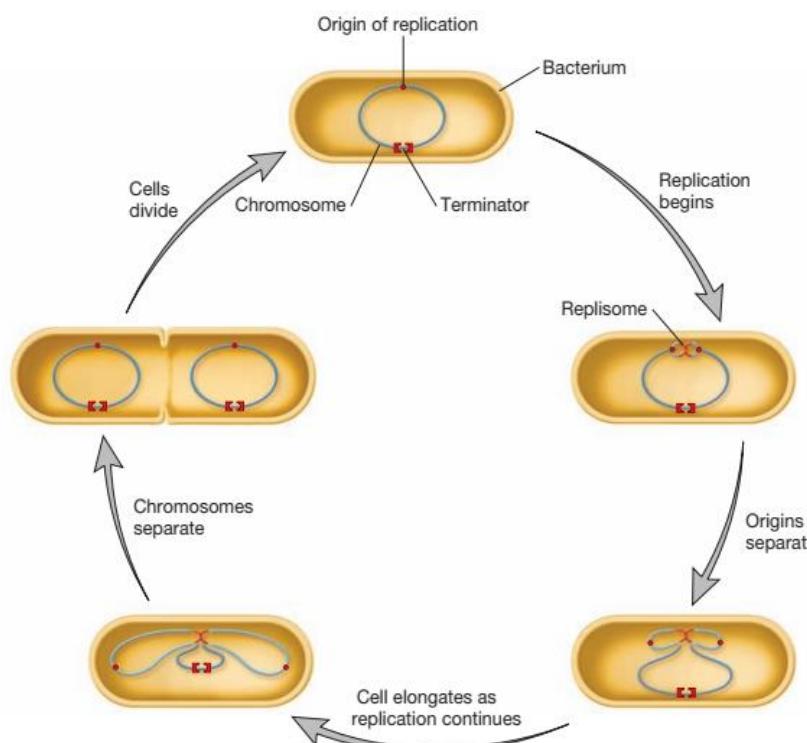
Recall that most prokaryotic chromosomes are circular. Each circular chromosome has a single site at which replication starts called the **origin of replication**, or simply the origin (**figure 6.3**).

Replication is completed at the **terminus**, which is located directly opposite the origin. In a newly formed *E. coli* cell, the chromosome is compacted and organized so that the origin and terminus are in opposite halves of the cell. Early in the cell cycle, the origin and terminus move to midcell and a group of proteins needed for DNA synthesis assemble to form the **replisome** at the origin. DNA replication proceeds in both directions from the origin and the parent DNA is thought to spool through the replisome, which remains relatively stationary. As progeny chromosomes are synthesized, the two newly formed origins move toward opposite ends of the cell, and the rest of the chromosome follows in an orderly fashion.

Although the process of DNA synthesis and movement seems rather straightforward, the mechanism by which chromosomes are partitioned to each daughter cell is not well understood. Surprisingly, a picture is emerging in which components of the cytoskeleton are involved. For many years, it was assumed that prokaryotes were too small for eukaryotic-like cytoskeletal structures. However, a protein called **MreB**, which is similar to eukaryotic actin, seems to be involved in several processes, including determining cell shape and chromosome movement. MreB polymerizes (that is to say, MreB units are linked together) to form a spiral around the inside periphery of the cell (**figure 6.4a**). One model suggests that the origin of each newly replicated chromosome associates with



**Figure 6.2 The Cell Cycle in *E. coli*.** A 60-minute interval between divisions has been assumed for purposes of simplicity (the actual time between cell divisions may be shorter). *E. coli* requires about 40 minutes to replicate its DNA and 20 minutes after termination of replication to prepare for division. The position of events on the time line is approximate and meant to show the general pattern of occurrences.



**Figure 6.3 Cell Cycle of Slow-Growing *E. coli*.** As the cell readies for replication, the origin migrates to the center of the cell and proteins that make up the replisome assemble. As replication proceeds, newly synthesized chromosomes move toward poles so that upon cytokinesis, each daughter cell inherits only one chromosome.

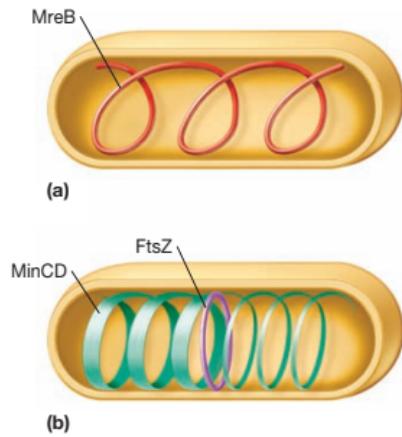
MreB, which then moves them to opposite poles of the cell. The notion that prokaryotic chromosomes may be actively moved to the poles is further suggested by the fact that if MreB is mutated so that it can no longer hydrolyze ATP, its source of energy, chromosomes fail to segregate properly.

### Cytokinesis

**Septation** is the process of forming a cross wall between two daughter cells. **Cytokinesis**, a term that has traditionally been used to describe the formation of two eukaryotic daughter cells, is now used to describe this process in prokaryotes as well. Septation is

divided into several steps: (1) selection of the site where the septum will be formed; (2) assembly of a specialized structure called the **Z ring**, which divides the cell in two by constriction; (3) linkage of the Z ring to the plasma membrane and perhaps components of the cell wall; (4) assembly of the cell wall-synthesizing machinery; and (5) constriction of the Z ring and septum formation.

The assembly of the Z ring is a critical step in septation, as it must be formed if subsequent steps are to occur. The **FtsZ protein**, a tubulin homologue found in most bacteria and many archaea, forms the Z ring. FtsZ, like tubulin, polymerizes to



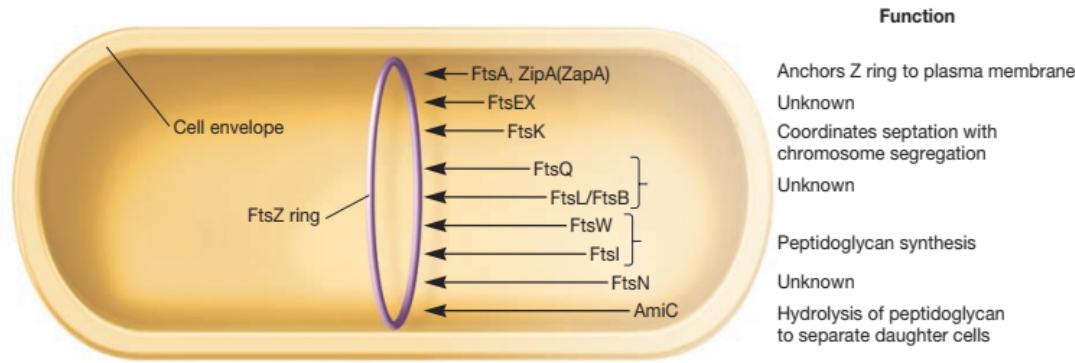
**Figure 6.4 Cytoskeletal Proteins Involved in Cytokinesis in Rod-Shaped Bacteria.** (a) The actin homolog MreB forms spiral filaments around the inside of the cell that help determine cell shape and may serve to move chromosomes to opposite cell poles. (b) The tubulin-like protein FtsZ assembles in the center of the cell to form a Z ring, which is essential for septation. MinCD, together with other Min proteins, oscillates from pole to pole, thereby preventing the formation of an off-center Z ring.

form filaments, which are thought to create the meshwork that constitutes the Z ring. Numerous studies show that the Z ring is very dynamic, with portions of the meshwork being exchanged constantly with newly formed, short FtsZ polymers from the cytosol. Another protein, called MinCD, is an inhibitor of Z-ring assembly. Like FtsZ, it is very dynamic, oscillating its position from one end of the cell to the other, forcing Z-ring formation only at the center of the cell (figure 6.4b). Once the Z-ring forms, the rest of the division machinery is constructed, as illustrated in figure 6.5. First one or more anchoring proteins link the Z ring to the cell membrane. Then the cell wall-synthesizing machinery is assembled. [The cytoplasmic matrix: The prokaryotic cytoskeleton \(section 3.3\)](#)

The final steps in division involve constriction of the Z ring, accompanied by invagination of the cell membrane and synthesis of the septal wall. Several models for Z-ring constriction have been proposed. One model holds that the FtsZ filaments are shortened by losing FtsZ subunits (i.e., depolymerization) at sites where the Z ring is anchored to the plasma membrane. This model is supported by the observation that Z rings of cells producing an excessive amount of FtsZ subunits fail to constrict.

#### DNA Replication in Rapidly Growing Cells

The preceding discussion of the cell cycle describes what occurs in slowly growing *E. coli* cells. In these cells, the cell cycle takes approximately 60 minutes to complete: 40 minutes for DNA replication and partitioning and about 20 minutes for septum formation and cytokinesis. However, *E. coli* can reproduce at a much more rapid rate, completing the entire cell cycle in about 20 minutes, despite the fact that DNA replication always requires at least 40 minutes. How can *E. coli* complete an entire cell cycle in 20 minutes when it takes 40 minutes to replicate its chromosome? *E. coli* accomplishes this by beginning a second round of DNA replication (and sometimes even a third or fourth round) before



**Figure 6.5 Formation of the Cell Division Apparatus in *E. coli*.** The cell division apparatus is composed of numerous proteins that are thought to assemble in the order shown. The process begins with the polymerization of FtsZ to form the Z ring. Then FtsA and ZipA (possibly ZapA in *Bacillus subtilis*) proteins anchor the Z ring to the plasma membrane. Although numerous proteins are known to be part of the cell division apparatus, the functions of relatively few are known.

the first round of replication is completed. Thus the progeny cells receive two or more replication forks, and replication is continuous because the cells are always copying their DNA.

1. What two pathways function during the prokaryotic cell cycle?
2. How does the prokaryotic cell cycle compare with the eukaryotic cell cycle? List two ways they are similar; list two ways they differ.

## 6.2 THE GROWTH CURVE

Binary fission and other cell division processes bring about an increase in the number of cells in a population. 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 6.6**).

### Lag Phase

When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs, so 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.

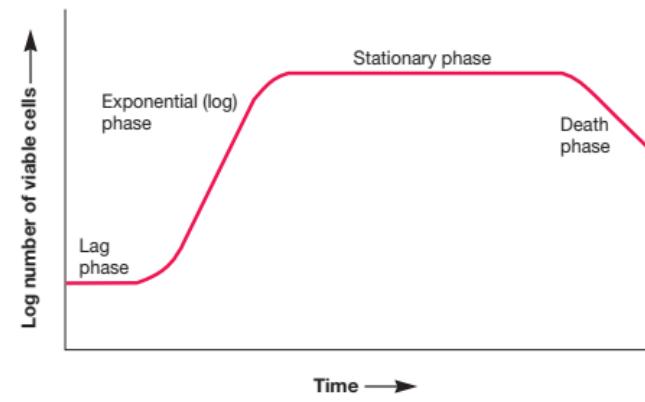
The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase. 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 6.6). 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.

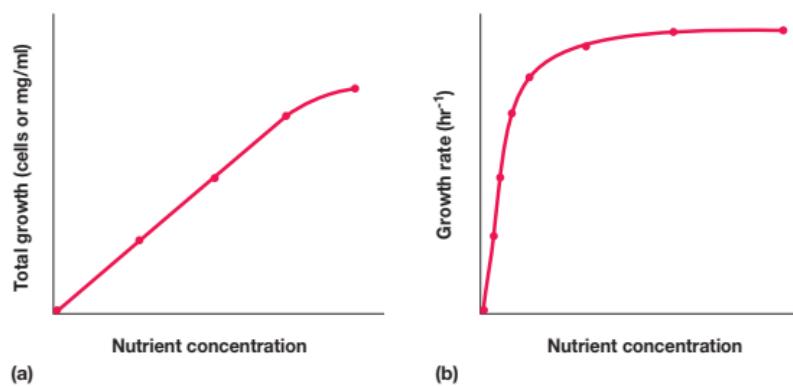
Exponential growth is **balanced growth**. That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, **unbalanced growth** results. This is growth during which the rates of synthesis of cell components vary relative to one another until a new balanced state is reached. Unbalanced growth is readily observed in two types of experiments: shift-up, where a culture is transferred from a nutritionally poor medium to a richer one; and shift-down, where a culture is transferred from a rich medium to a poor one. In a shift-up experiment, there is a lag while 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. In a shift-down experiment, there is a lag in growth because cells 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. 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 6.7a**). This is the basis of microbiological assays for vitamins and other growth



**Figure 6.6** Microbial Growth Curve in a Closed System. The four phases of the growth curve are identified on the curve and discussed in the text.

**Figure 6.7 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.



factors. The rate of growth also increases with nutrient concentration (figure 6.7b), but in a hyperbolic manner much like that seen with many enzymes (see figure 8.18). 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. [Uptake of nutrients by the cell \(section 5.6\)](#)

### Stationary Phase

Because this is a closed system, eventually population growth ceases and the growth curve becomes horizontal (figure 6.6). 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; protist cultures often have 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 but remain metabolically active.

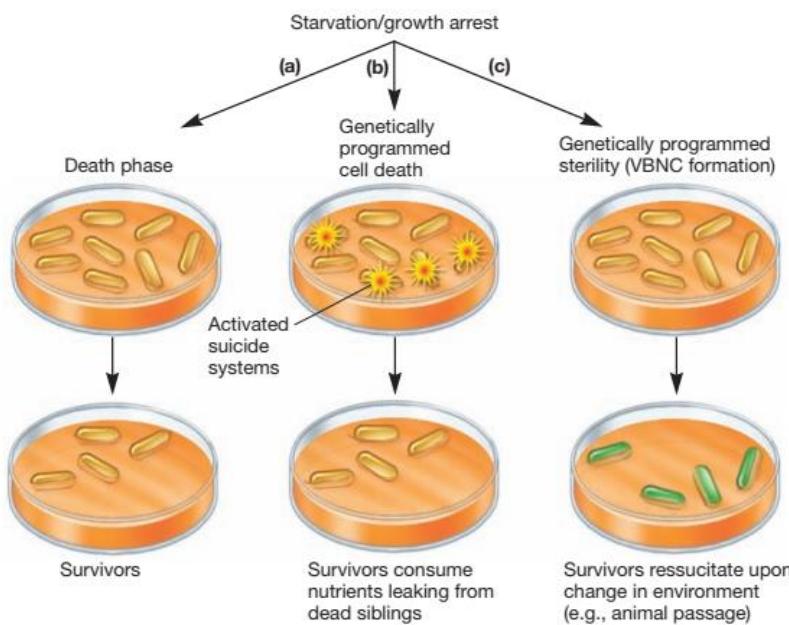
Microbial populations enter the stationary phase for several reasons. One obvious factor is nutrient limitation; if an essential nutrient is severely depleted, population growth will slow. Aerobic organisms often are limited by  $O_2$  availability. Oxygen is not very soluble and may be depleted so quickly 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 because many environments have low nutrient levels. Prokaryotes have evolved a number of strategies to survive starvation. 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 crosslinking and cell wall strength. The Dps (DNA-binding protein from starved cells) protein protects DNA. Chaperone proteins 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. There is even evidence that *Salmonella enterica* serovar Typhimurium (*S. typhimurium*), and some other bacterial pathogens become more virulent when starved. Clearly, these considerations are of great practical importance in medical and industrial microbiology.

### Senescence and Death

For many years, the decline in viable cells following stationary cells was described simply as the "death phase." It was assumed that detrimental environmental changes like nutrient deprivation and the buildup of toxic wastes caused irreparable harm resulting in loss of viability. That is, even when bacterial cells were transferred to fresh medium, no cellular growth was observed. Because loss of viability was often not accompanied by a loss in total cell number, it was assumed that cells died but did not lyse.

This view is currently under debate. There are two alternative hypotheses (figure 6.8). Some microbiologists believe starving cells that show an exponential decline in density have not irreversibly lost their ability to reproduce. Rather, they suggest that microbes are temporarily unable to grow, at least under the labora-



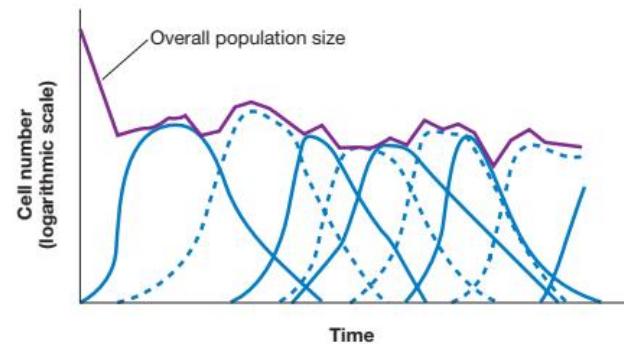
**Figure 6.8 Loss of Viability.** (a) It has long been assumed that as cells leave stationary phase due to starvation or toxic waste accumulation, the exponential decline in culturability is due to cellular death. (b) Some believe that a fraction of a microbial population dies due to activation of programmed cell death genes. The nutrients that are released by dying cells supports the growth of other cells. (c) The viable but nonculturable (VBNC) hypothesis posits that when cells are starved, they become temporarily nonculturable under laboratory conditions. When exposed to appropriate conditions, some cells will regain the capacity to reproduce.

tory conditions used. This phenomenon, in which the cells are called **viable but nonculturable (VBNC)**, is thought to be the result of a genetic response triggered in starving, stationary phase cells. Just as some bacteria form spores as a survival mechanism, it is argued that others are able to become dormant without changes in morphology (figure 6.8c). Once the appropriate conditions are available (for instance, a change in temperature or passage through an animal), VBNC microbes resume growth. VBNC microorganisms could pose a public health threat, as many assays that test for food and drinking water safety are culture-based.

The second alternative to a simple death phase is **programmed cell death** (figure 6.8b). In contrast to the VBNC hypothesis whereby cells are genetically programmed to survive, programmed cell death predicts that a fraction of the microbial population is genetically programmed to commit suicide. In this case, nonculturable cells are dead (as opposed to nonculturable) and the nutrients that they leak enable the eventual growth of those cells in the population that did not initiate suicide. The dying cells are thus altruistic—that is to say, they sacrifice themselves for the benefit of the larger population.

#### Phase of Prolonged Decline

Long-term growth experiments reveal that an exponential decline in viability is sometimes replaced by a gradual decline in the number of culturable cells. This decline can last months to years



**Figure 6.9 Prolonged Decline in Growth.** Instead of a distinct death phase, successive waves of genetically distinct subpopulations of microbes better able to use the released nutrients and accumulated toxins survive. Each successive solid or dashed curve represents the growth of a new subpopulation.

(figure 6.9). During this time the bacterial population continually evolves so that actively reproducing cells are those best able to use the nutrients released by their dying brethren and best able to tolerate the accumulated toxins. This dynamic process is marked by successive waves of genetically distinct variants. Thus natural selection can be witnessed within a single culture vessel.

### 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 are applied in industry. The quantitative aspects of exponential phase growth discussed here apply to microorganisms that divide by binary fission.

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 6.10**).

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,

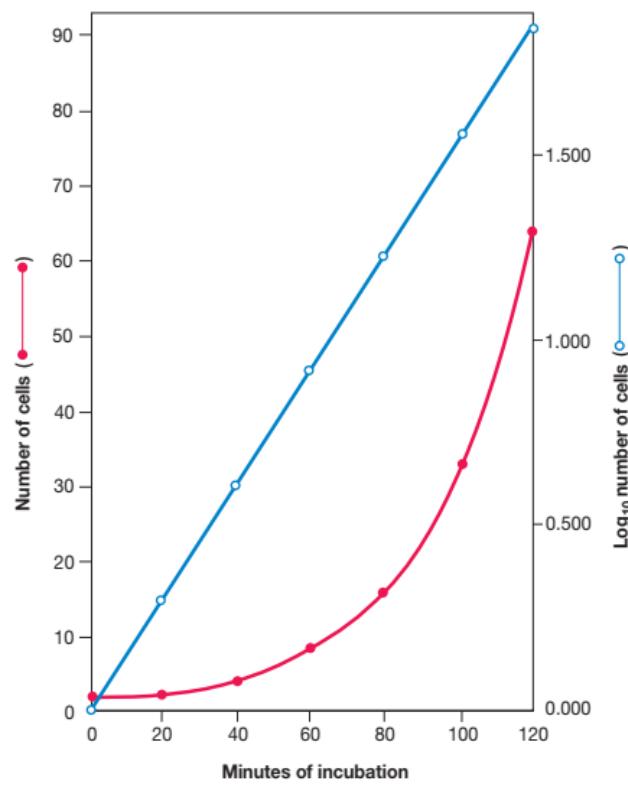
$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

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

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the **mean growth rate constant ( $k$ )**.

Table 6.1 An Example of Exponential Growth				
Time <sup>a</sup>	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

<sup>a</sup>The hypothetical culture begins with one cell having a 20-minute generation time.



**Figure 6.10 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.

This is the number of generations per unit time, often expressed as the generations per hour.

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

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

$$N_t = 2N_0.$$

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

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

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

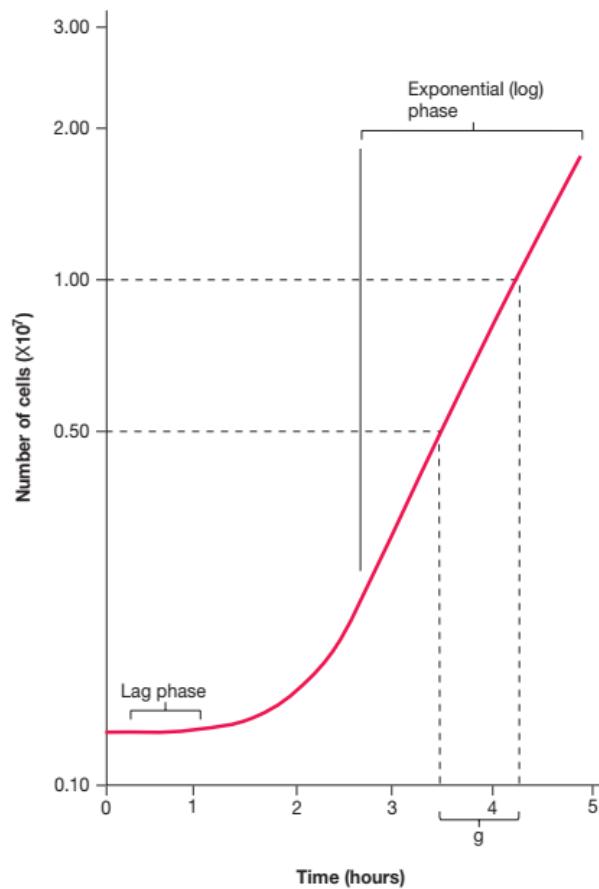
The mean generation time is the reciprocal of the mean growth rate constant.

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

The mean generation time ( $g$ ) can be determined directly from a semilogarithmic plot of the growth data (figure 6.11) 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.

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

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



**Figure 6.11 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.

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

<sup>a</sup> Generation times differ depending on the growth medium and environmental conditions used.

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 eukaryotic microorganisms (table 6.2). Generation times in nature are usually much longer than in culture.

1. Define growth. Describe the four phases of the growth curve in a closed system and discuss the causes of each.
2. Why might a culture have a long lag phase after inoculation? Why would cells that are vigorously growing when inoculated into fresh culture medium have a shorter lag phase than those that have been stored in a refrigerator?
3. List two physiological changes that are observed in stationary cells. How do these changes impact the organism's ability to survive?
4. Define balanced growth and unbalanced growth. Why do shift-up and shift-down experiments cause cells to enter unbalanced growth?
5. Define the generation or doubling time and the mean growth rate constant. Calculate the mean growth rate and generation time of a culture that increases in the exponential phase from  $5 \times 10^2$  to  $1 \times 10^8$  in 12 hours.

6. Suppose the generation time of a bacterium is 90 minutes and the initial number of cells in a culture is  $10^3$  cells at the start of the log phase. How many bacteria will there be after 8 hours of exponential growth?
7. What effect does increasing a limiting nutrient have on the yield of cells and the growth rate?
8. Contrast and compare the viable but nonculturable status of microbes with that of programmed cell death as a means of responding to starvation.

### 6.3 MEASUREMENT OF MICROBIAL GROWTH

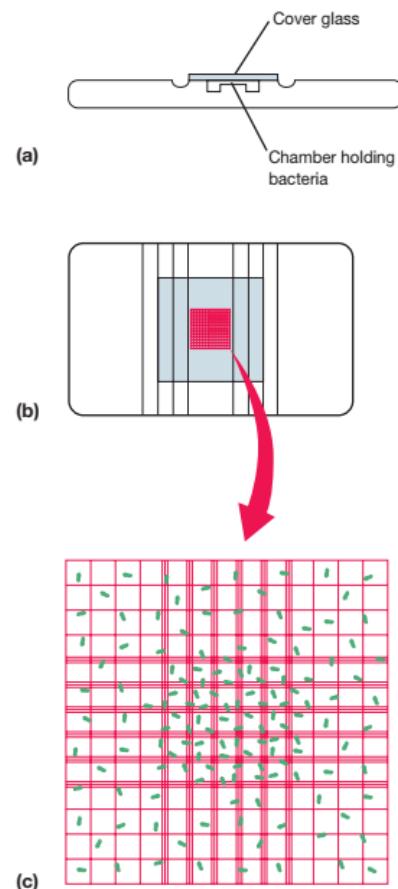
There are many ways to measure microbial growth to determine growth rates and generation times. Either population number or mass may be followed because growth leads to increases in both. Here the most commonly employed techniques for growth measurement are examined briefly and the advantages and disadvantages 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 prokaryotes; hemacytometers can be used for both prokaryotes and eukaryotes. These specially designed slides have chambers of known depth with an etched grid on the chamber bottom (**figure 6.12**). Prokaryotes are more easily counted in these chambers if they are stained, or when a phase-contrast or a fluorescence microscope is employed. The number of microorganisms in a sample can be calculated by taking into account the chamber's volume and any sample dilutions required. One disadvantage to the technique is that the microbial population must be fairly large for accuracy because such a small volume is sampled.

Larger microorganisms such as protists and yeasts can be directly counted with electronic counters such as the **Coulter Counter**, although more recently the **flow cytometer** is increasingly used. The microbial suspension is forced through a small hole or orifice in the Coulter Counter. 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. **Identification of microorganisms from specimens (section 35.2)**

The number of bacteria in aquatic samples is frequently determined from direct counts after the bacteria have been trapped on special membrane filters. In the **membrane filter technique**, the sample is first filtered through a black polycarbonate membrane filter. Then the bacteria are stained with a fluorescent dye such as acridine orange or the DNA stain DAPI, and observed microscopically. The stained cells are easily observed against the black



**Figure 6.12** 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  $\times 400$  to  $\times 500$  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  $1 \text{ mm}^2$ , the total number of bacteria in  $1 \text{ mm}^2$  of the chamber is  $(\text{number/square})(25 \text{ squares})$ . The chamber is  $0.02 \text{ mm}$  deep and therefore,

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

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

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

background of the membrane filter and can be counted when viewed with an epifluorescence microscope. **The light microscope: The fluorescence microscope (section 2.2)**

Traditional methods for directly counting microbes in a sample usually yield cell densities that are much higher than the plat-

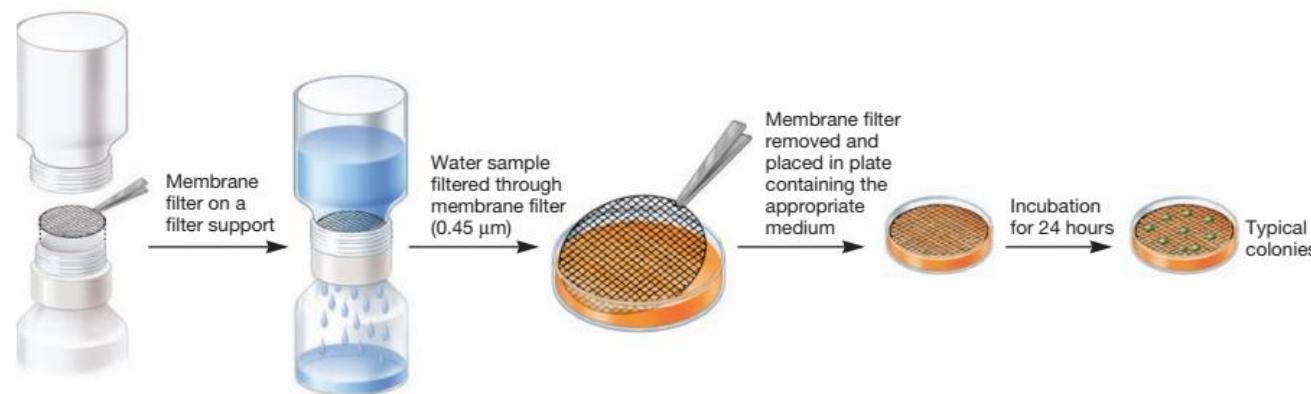
ing methods described next because direct counting procedures do not distinguish dead cells from live cells. Newer methods for direct counts avoid this problem. Commercial kits that use fluorescent reagents to stain live and dead cells differently are now available, making it possible to directly count the number of live and dead microorganisms in a sample (see figures 2.13a and 27.16).

Several **plating methods** can be used to determine the number of viable microbes in a sample. These are referred to as **viable counting methods** because they count only those cells that are alive and able to reproduce. Two commonly used procedures are the **spread-plate technique** and the **pour-plate technique**. In both of these methods, 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  $1 \times 10^{-6}$  dilution yielded 150 colonies, the original sample contained around  $1.5 \times 10^8$

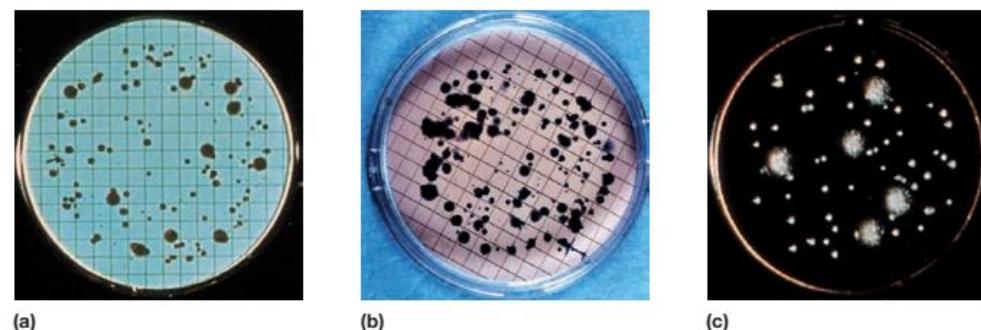
cells per ml. Usually the count is made more accurate by use of a special colony counter. In this way the spread-plate and pour-plate techniques may be used to find the number of microorganisms in a sample. [Isolation of pure cultures: The spread plate and streak plate; The pour plate \(section 5.8\)](#)

Another commonly used plating method first traps bacteria in aquatic samples on a membrane filter. The filter is then placed on an agar medium or on a pad soaked with liquid media (**figure 6.13**) 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 6.14**). This technique is especially useful in analyzing water purity. [Water purification and sanitary analysis \(section 41.1\)](#)

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



**Figure 6.13 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.



**Figure 6.14 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) Wort agar for the culture of yeasts and molds.

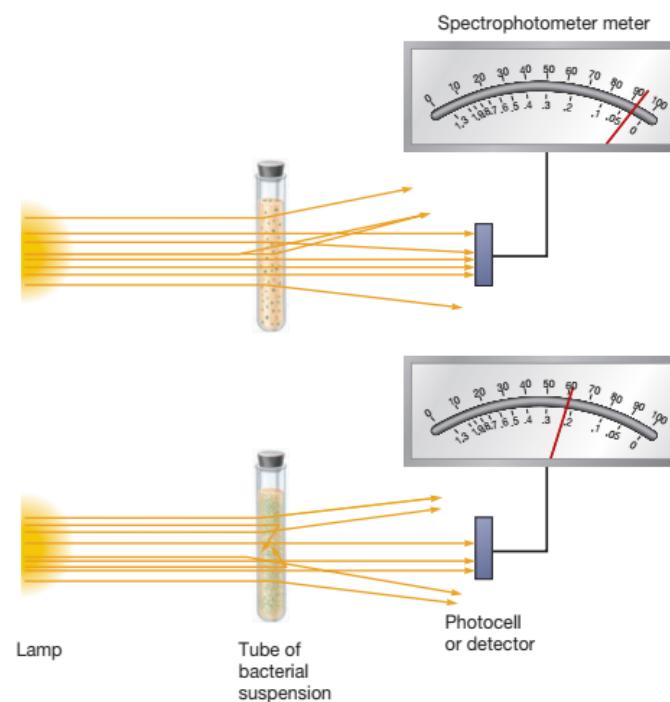
broken up and the microorganisms well dispersed. Because it is not possible to be absolutely certain that each colony arose from an individual cell, the results are often expressed in terms of **colony forming units (CFU)** rather than the number of microorganisms. The samples should yield between 30 and 300 colonies for most accurate counting. 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.

### 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 filamentous 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.

**Spectrophotometry** can also be used to measure cell mass. These methods are more rapid and sensitive. They depend on the fact that microbial cells scatter light that strikes 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 cell concentration at low absorbance levels (figure 6.15). Thus population growth can be easily measured 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 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 and cyanobacterial populations, and the quantity of ATP can be used to estimate the amount of living microbial mass.



**Figure 6.15 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.

1. Briefly describe each technique by which microbial population numbers may be determined and give its advantages and disadvantages.
2. When using direct cell counts to follow the growth of a culture, it may be difficult to tell when the culture enters the phase of senescence and death. Why?
3. Why are plate count results expressed as colony forming units?

#### 6.4 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.

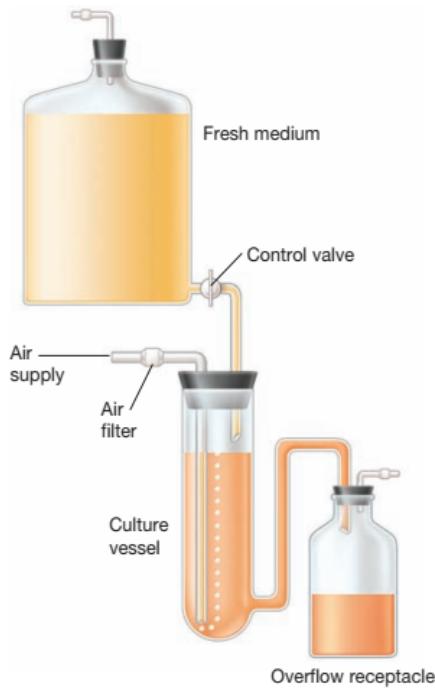
##### The Chemostat

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 6.16). The culture medium for a chemostat possesses an essential nutrient (e.g., an amino acid) in limiting quantities. Because one nutrient is limiting, 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).

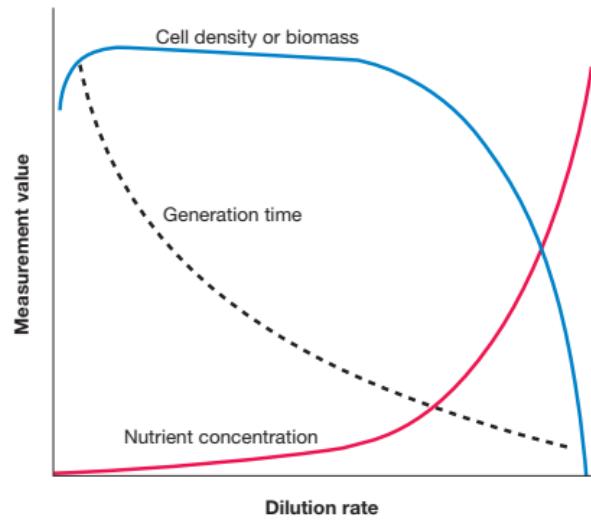
$$D = f/V$$

For example, if  $f$  is 30 ml/hr and  $V$  is 100 ml, the dilution rate is 0.30 hr<sup>-1</sup>.

Both the microbial population level and the generation time are related to the dilution rate (figure 6.17). The microbial population density remains unchanged over a wide range of dilution rates. The generation time decreases (i.e., the rate of growth increases) 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. This occurs because fewer microorganisms are present to consume the limiting nutrient.



**Figure 6.16 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 6.17 Chemostat Dilution Rate and Microbial Growth.** The effects of changing the dilution rate in a chemostat.

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 6.7b). 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 reproduction. 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 contains all nutrients in excess. That is, none of the nutrients is limiting. 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 (chapters 40 and 41, respectively).

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?
4. How is the rate of growth of a microbial population controlled in a chemostat? In a turbidostat?

## 6.5 THE INFLUENCE OF ENVIRONMENTAL FACTORS ON GROWTH

As we have seen, 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. Prokaryotes are present anywhere life can exist. Many habitats in which prokary-

otes thrive would kill most other organisms. Prokaryotes such as *Bacillus infernus* are even 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. It is important to note that for most environmental factors, a range of levels supports growth of a microbe. For example, a microbe might exhibit optimum growth at pH 7, but will grow, though not optimally, at pH values down to pH 6 (its pH minimum) and up to pH 8 (its pH maximum). Furthermore, outside this range, the microbe might cease reproducing but will remain viable for some time. Clearly, each microbe must possess adaptations that allow it to adjust its physiology within its preferred range, and it may also have adaptations that protect it in environments outside this range. These adaptations will also be discussed in this section.

### 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. Conversely if it is placed in a hypertonic solution (one with a higher osmotic concentration), water will flow out of the cell. In microbes that have cell walls (i.e., most prokaryotes, fungi, and algae), the membrane shrinks away from the cell wall—a process called **plasmolysis**. Dehydration of the cell in hypertonic environments may damage the cell membrane and cause the cell to become metabolically inactive.

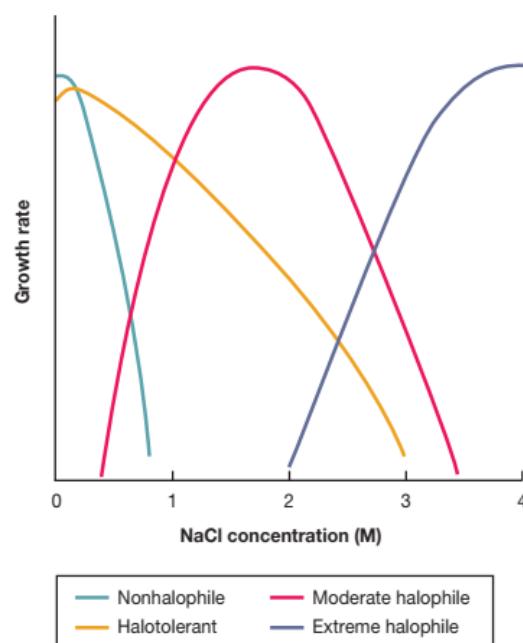
It is important, then, that microbes be able to respond to changes in the osmotic concentrations of their environment. For instance, microbes in hypotonic environments can reduce the osmotic concentration of their cytoplasm. This can be achieved by the use of inclusion bodies. Some bacteria and archaea also have mechanosensitive (MS) channels in their plasma membrane. In a hypotonic environment, the membrane stretches due to an increase in hydrostatic pressure and cellular swelling. MS channels then open and allow solutes to leave. Thus they can act as escape valves to protect cells from bursting. Since many protists do not have a cell wall, they must use contractile vacuoles (see figures 25.5 and 25.17b) to expel excess water. Many microorganisms, whether in hypotonic or hypertonic environments, 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 do not interfere with metabolism and growth when at high intracellular concentrations. Most prokaryotes increase their

Table 6.3 Microbial Responses to Environmental Factors		
Descriptive Term	Definition	Representative Microorganisms
<b>Solute and Water Activity</b>		
Osmotolerant	Able to grow over wide ranges of water activity or osmotic concentration	<i>Staphylococcus aureus, Saccharomyces rouxii</i>
Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	<i>Halobacterium, Dunaliella, Ectothiorhodospira</i>
<b>pH</b>		
Acidophile	Growth optimum between pH 0 and 5.5	<i>Sulfolobus, Picrophilus, Ferroplasma, Acontium, Cyanidium caldarium</i>
Neutrophile	Growth optimum between pH 5.5 and 8.0	<i>Escherichia, Euglena, Paramecium</i>
Alkalophile	Growth optimum between pH 8.0 and 11.5	<i>Bacillus alcalophilus, Natronobacterium</i>
<b>Temperature</b>		
Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	<i>Bacillus psychrophilus, Chlamydomonas nivalis</i>
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	<i>Listeria monocytogenes, Pseudomonas fluorescens</i>
Mesophile	Has growth optimum around 20–45°C	<i>Escherichia coli, Neisseria gonorrhoeae, Trichomonas vaginalis</i>
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	<i>Geobacillus stearothermophilus, Thermus aquaticus, Cyanidium caldarium, Chaetomium thermophile</i>
Hyperthermophile	Has an optimum between 80 and about 113°C	<i>Sulfolobus, Pyrococcus, Pyrodictium</i>
<b>Oxygen Concentration</b>		
Obligate aerobe	Completely dependent on atmospheric O <sub>2</sub> for growth	<i>Micrococcus luteus, Pseudomonas, Mycobacterium; most protists and fungi</i>
Facultative anaerobe	Does not require O <sub>2</sub> for growth, but grows better in its presence	<i>Escherichia, Enterococcus, Saccharomyces cerevisiae</i>
Aerotolerant anaerobe	Grows equally well in presence or absence of O <sub>2</sub>	<i>Streptococcus pyogenes</i>
Obligate anaerobe	Does not tolerate O <sub>2</sub> and dies in its presence	<i>Clostridium, Bacteroides, Methanobacterium, Treponomas agilis</i>
Microaerophile	Requires O <sub>2</sub> levels below 2–10% for growth and is damaged by atmospheric O <sub>2</sub> levels (20%)	<i>Campylobacter, Spirillum volutans, Treponema pallidum</i>
<b>Pressure</b>		
Barophilic	Growth more rapid at high hydrostatic pressures	<i>Photobacterium profundum, Shewanella benthica, Methanocaldococcus jannaschii</i>

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. Photosynthetic protists 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. [The cytoplasmic matrix: Inclusion bodies \(section 3.3\)](#)

Some microbes are adapted to extreme hypertonic environments. **Halophiles** grow optimally in the presence of NaCl or other salts at a concentration above about 0.2 M ([figure 6.18](#)). Extreme halophiles have adapted so completely to hypertonic, saline conditions that they require high levels of sodium chloride to grow—concentrations between about 2 M and saturation (about 6.2 M). 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 prokaryotes accumulate enormous quantities of potassium in order to remain hypertonic to their environment; the internal potassium concentration may reach 4 to 7 M. Furthermore, their enzymes, ribosomes, and transport proteins require high potassium levels 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 disintegrate. Extreme halophiles 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. [Phylum Euryarchaeota: The Halobacteria \(section 20.3\)](#)



**Figure 6.18 The Effects of Sodium Chloride on Microbial Growth.** Four different patterns of microbial dependence on NaCl concentration are depicted. The curves are only illustrative and are not meant to provide precise shapes or salt concentrations required for growth.

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* is halotolerant (figure 6.18) and can be cultured in media containing 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 photosynthetic protist *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. [Controlling food spoilage \(section 40.3\)](#)

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).

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

The pH scale extends from pH 0.0 (1.0 M H<sup>+</sup>) to pH 14.0 (1.0 × 10<sup>-14</sup> M H<sup>+</sup>), and each pH unit represents a tenfold change in hydrogen ion concentration. [Figure 6.19](#) shows that the habitats in which microorganisms grow vary widely—from pH 0 to 2 at the acidic 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 8.0 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 protists are neutrophiles. Most fungi prefer more acidic surroundings, about pH 4 to 6; photosynthetic protists also seem to favor slight acidity. Many archaea are acidophiles. For example, the archaeon *Sulfolobus acidocaldarius* is a common inhabitant of acidic hot springs; it grows 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.

Table 6.4   Approximate Lower $a_w$ Limits for Microbial Growth				
Water Activity	Environment	Prokaryotes	Fungi	Photosynthetic protists
1.00—Pure water	Blood Plant wilt Seawater	Vegetables, meat, fruit	Most gram-negative bacteria and other nonhalophiles	
0.95	Bread		Basidiomycetes	Most genera
0.90	Ham	Most cocci, <i>Bacillus</i>	<i>Fusarium</i> <i>Mucor, Rhizopus</i> Ascomycetous yeasts	
0.85	Salami	<i>Staphylococcus</i>	<i>Saccharomyces rouxii</i> (in salt)	
0.80	Preserves		<i>Penicillium</i>	
0.75	Salt lakes Salted fish	<i>Halobacterium</i> <i>Actinospora</i>	<i>Aspergillus</i>	<i>Dunaliella</i>
0.70	Cereals, candy, dried fruit		<i>Aspergillus</i>	
0.60	Chocolate Honey Dried milk		<i>Saccharomyces rouxii</i> (in sugars) <i>Xeromyces bisporus</i>	
0.55—DNA disordered				

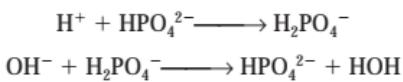
Adapted from A. D. Brown, "Microbial Water Stress," in *Bacteriological Reviews*, 40(4):803–846 1976. Copyright © 1976 by the American Society for Microbiology. Reprinted by permission.

Drastic variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Most prokaryotes 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.

Microorganisms respond to external pH changes using mechanisms that maintain a neutral cytoplasmic pH. Several mechanisms for adjusting to small changes in external pH have been proposed. The plasma membrane is impermeable to protons. Neutrophiles appear to exchange potassium for protons using an antiport transport system. 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. However, if the external pH becomes too acidic, other mechanisms come into play. When the pH drops below about 5.5 to 6.0, *Salmonella enterica* serovar 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, chaperone proteins such as acid shock proteins and heat shock proteins are synthesized. These prevent the acid denaturation of proteins and aid in the refolding of denatured proteins. [Uptake of nutrients by the cell \(section 5.6\); Translation: Protein folding and molecular chaperones \(section 11.8\)](#)

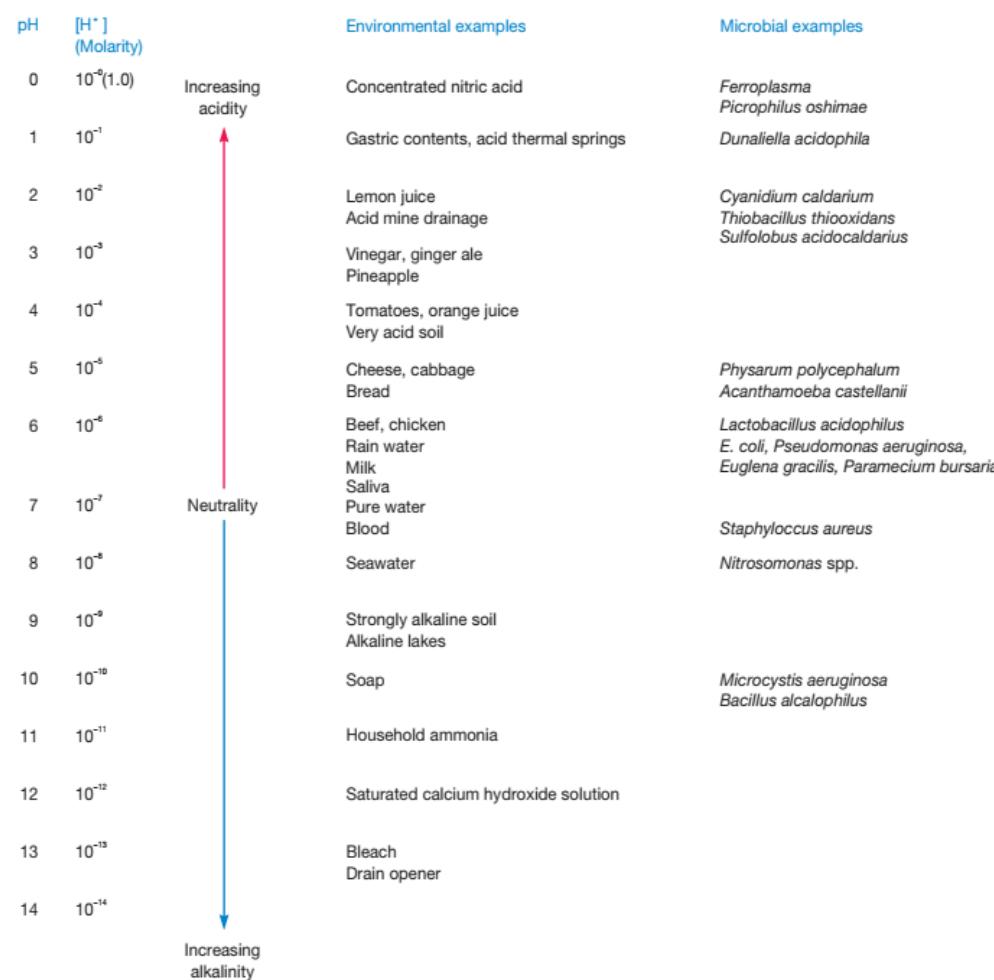
Microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste products. Fermentative microorganisms form organic acids from carbohydrates, whereas chemolithotrophs like *Thiobacillus* oxidize reduced sulfur components to sulfuric acid. Other microorganisms make their environment more alkaline by generating ammonia through amino acid degradation. [Fermentation \(section 9.7\); Chemolithotrophy \(section 9.11\)](#)

Because microorganisms change the pH of their surroundings, 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 ( $\text{H}_2\text{PO}_4^-$ ) and its conjugate base ( $\text{HPO}_4^{2-}$ ).



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.

1. Define pH, acidophile, neutrophile, and alkalophile.
2. Classify each of the following organisms as an alkalophile, a neutrophile, or an acidophile: *Staphylococcus aureus*, *Microcystis aeruginosa*, *Sulfolobus acidocaldarius*, and *Pseudomonas aeruginosa*. Which might be pathogens? Explain your choices.



**Figure 6.19 The pH Scale.** The pH scale and examples of substances with different pH values. The microorganisms are placed at their growth optima.

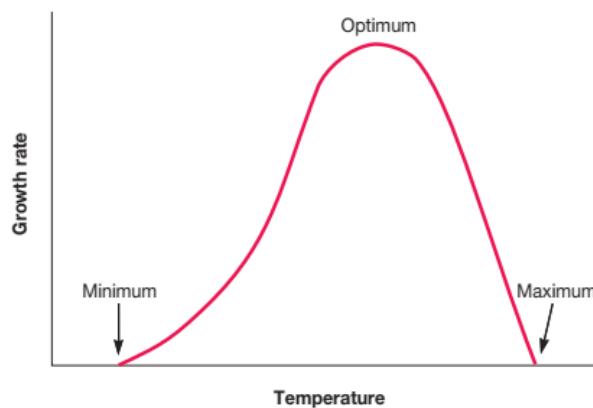
3. Describe the mechanisms microbes use to maintain a neutral pH. Explain how extreme pH values might harm microbes.
4. How do microorganisms change the pH of their environment? How does the microbiologist minimize this effect when culturing microbes in the lab?

### Temperature

Environmental temperature profoundly affects microorganisms, like all other organisms. Indeed, microorganisms are particularly susceptible because their temperature varies with that of the external environment. A most important factor influencing the effect of temperature on growth is the temperature sensitivity of enzyme-catalyzed reactions. Each enzyme has a temperature at which it functions optimally (see figure 8.19b). At some temperature below the optimum, it ceases to be catalytic. As the temperature rises from this low temperature, the rate of catalysis increases to that observed for the optimal temperature. The ve-

locity of the reaction will roughly double for every  $10^\circ\text{C}$  rise in temperature. When all enzymes in a microbe are considered together, as the rate of each reaction increases, metabolism as a whole becomes more active, and the microorganism grows faster. However, 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. Temperature also has a significant effect on microbial membranes. At very low temperatures, membranes solidify. At high temperatures, the lipid bilayer simply melts and disintegrates. In summary, when organisms are above their 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.

Because of these opposing temperature influences, microbial growth has a fairly characteristic temperature dependence with distinct **cardinal temperatures**—minimum, optimum, and max-



**Figure 6.20 Temperature and Growth.** The effect of temperature on growth rate.

imum growth temperatures (**figure 6.20**). 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, *Critchidia fasciculata*, a flagellated protist living in the gut of mosquitoes, 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.

The cardinal temperatures vary greatly between microorganisms (**table 6.5**). Optima usually range from 0°C to 75°C, whereas microbial growth occurs at temperatures extending from less than -20°C to over 120°C. Some archaea can even grow at 121°C (250°F), the temperature normally used in autoclaves (**Microbial Diversity and Ecology 6.1**). 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 temperatures. The upper limit for protists is around 50°C. Some fungi can grow at temperatures as high as 55 to 60°C. Prokaryotes 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.

Microorganisms such as those listed in table 6.5 can be placed in one of five classes based on their temperature ranges for growth (**figure 6.21**).

1. **Psychrophiles** grow well at 0°C and have an optimum growth temperature of 15°C or lower; the maximum is around

**Table 6.5 Temperature Ranges for Microbial Growth**

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

<sup>a</sup>ND, no data.

10°C. They are readily isolated from Arctic and Antarctic habitats; because 90% of the ocean is 5°C or colder, it constitutes an enormous habitat for psychrophiles. The psychophilic protist *Chlamydomonas nivalis* can actually turn a snowfield or glacier pink with its bright red spores. Psychrophiles are widespread among bacterial taxa and are found in such genera as *Pseudomonas*, *Vibrio*, *Alcaligenes*, *Bacillus*, *Arthrobacter*,

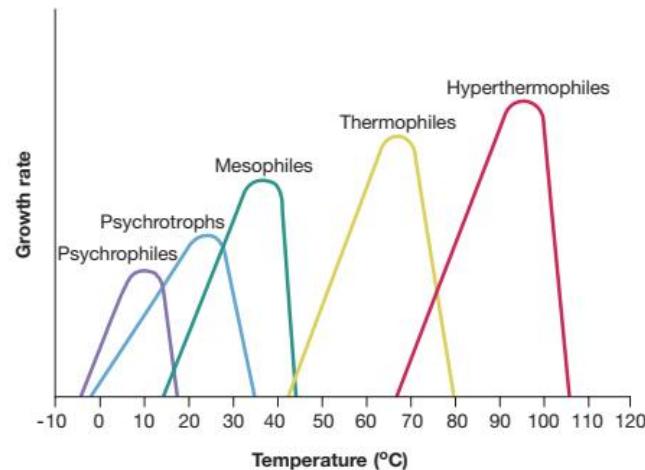
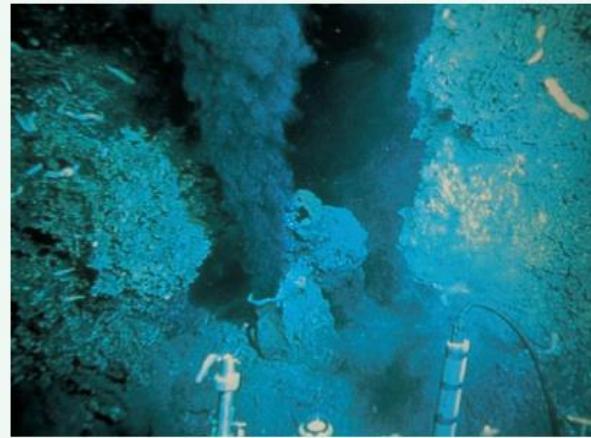


## Microbial Diversity & Ecology

### 6.1 Life above 100°C

Until recently the highest reported temperature for prokaryotic growth was 105°C. It seemed that the upper temperature limit for life was about 100°C, the boiling point of water. Now thermophilic prokaryotes have been reported growing in sulfide chimneys or "black smokers," located along rifts and ridges on the ocean floor, that spew sulfide-rich super-heated vent water with temperatures above 350°C (see **Box figure**). Evidence has been presented that these microbes can grow and reproduce at 121°C and can survive temperatures to 130°C for up to 2 hours. 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 prokaryotes 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. [The polymerase chain reaction \(section 14.3\)](#)



**Figure 6.21 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.

*Moritella*, *Photobacterium*, and *Shewanella*. A psychrophilic archaeon, *Methanogenium*, has 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 as described in chapter 40.
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 because 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 prokaryotes although a few photosynthetic protists 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 many ways. They have more heat-stable enzymes and protein synthesis systems, which function properly at high temperatures. These proteins are stable for a variety of reasons. Heat-stable proteins have highly organized, hydrophobic interiors; more hydrogen bonds and other noncovalent bonds strengthen the structure. Larger quantities of amino acids such as proline

also make the polypeptide chain less flexible. In addition, the proteins are stabilized and aided in folding by special chaperone proteins. There is evidence that in thermophilic bacteria, DNA is stabilized by special histonelike proteins. Their membrane lipids are also quite temperature stable. They tend to be more saturated, more branched, and of higher molecular weight. This increases the melting points of membrane lipids. Archaeal thermophiles have membrane lipids with ether linkages, which protect the lipids from hydrolysis at high temperatures. Sometimes archaeal lipids actually span the membrane to form a rigid, stable monolayer. [Proteins \(appendix D\); Prokaryotic cell membranes \(section 3.2\)](#)

- As mentioned previously, a few thermophiles can grow at 90°C or above and some have maxima above 100°C. Prokaryotes 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.

- What are cardinal temperatures?
- Why does the growth rate rise with increasing temperature and then fall again at higher temperatures?
- Define psychrophile, psychrotroph, mesophile, thermophile, and hyperthermophile.
- What metabolic and structural adaptations for extreme temperatures do psychrophiles and thermophiles have?

### Oxygen Concentration

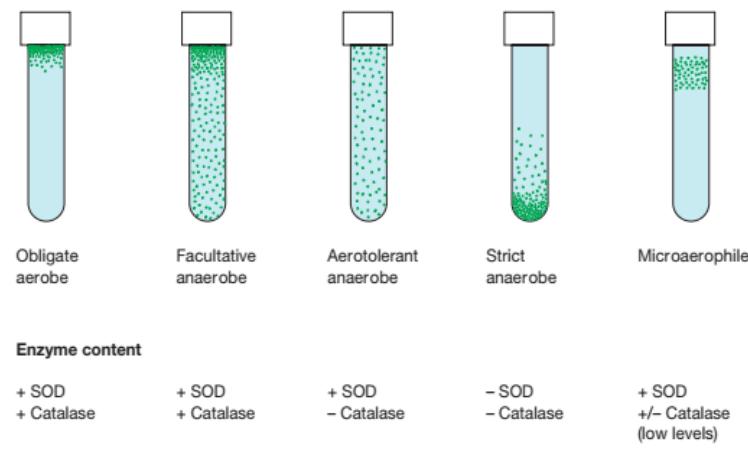
The importance of oxygen to the growth of an organism correlates with its metabolism—in particular, with the processes it uses to conserve the energy supplied by its energy source. Almost all energy-conserving metabolic processes involve the movement of electrons through an electron transport system. For chemotrophs, an externally supplied terminal electron acceptor is critical to the functioning of the electron transport system. The nature of the terminal electron acceptor is related to an organism's oxygen requirement.

An organism able to grow in the presence of atmospheric O<sub>2</sub> is an **aerobe**, whereas one that can grow in its absence is an **anaerobe**. Almost all multicellular organisms are completely dependent on atmospheric O<sub>2</sub> 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<sub>2</sub> in the synthesis of sterols and unsaturated fatty acids. **Facultative anaerobes** do not require O<sub>2</sub> for growth but grow better in its presence. In the presence of oxygen they use aerobic respiration. **Aerotolerant anaerobes** such as *Enterococcus faecalis* simply ignore O<sub>2</sub> and grow equally well whether it is present or not. In contrast, **strict** or **obligate anaerobes** (e.g., *Bacteroides*, *Fusobacterium*, *Clostridium pasteurianum*, *Methanococcus*, *Neocallimastix*) do not tolerate O<sub>2</sub> at all and die in its presence. Aerotolerant and strict anaerobes cannot generate energy through aerobic respiration and must employ fermentation or anaerobic respiration for this purpose. Finally, there are aerobes such as *Campylobacter*, called **microaerophiles**, that are damaged by the normal atmospheric level of O<sub>2</sub> (20%) and require O<sub>2</sub> levels below the range of 2 to 10% for growth. The nature of bacterial O<sub>2</sub> 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<sub>2</sub> levels (**figure 6.22**). [Oxidation-reduction reactions, electron carriers, and electron transport systems \(section 8.6\); Aerobic respiration \(section 9.2\); Anaerobic respiration \(section 9.6\); Fermentation \(section 9.7\)](#)

A microbial group may show more than one type of relationship to O<sub>2</sub>. All five types are found among the prokaryotes and protozoa. Fungi are normally aerobic, but a number of species—particularly among the yeasts—are facultative anaerobes. Photosynthetic protists are almost always obligate aerobes. It should be noted that the ability to grow in both oxic and anoxic environments provides considerable flexibility and is an ecological advantage.

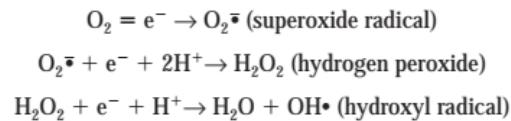
Although obligate anaerobes are killed by O<sub>2</sub>, they may be recovered from habitats that appear to be oxic. In such cases they associate with facultative anaerobes that use up the available O<sub>2</sub> and thus make the growth of strict anaerobes possible. For example, the strict anaerobe *Bacteroides gingivalis* lives in the mouth where it grows in the anoxic crevices around the teeth.

**Figure 6.22** Oxygen and Bacterial Growth. 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 oxic. The oxygen content of the medium decreases with depth until the medium becomes anoxic toward the bottom of the tube. The presence and absence of the enzymes superoxide dismutase (SOD) and catalase for each type are shown.



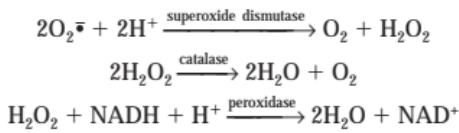
These different relationships with O<sub>2</sub> are due to several factors, including the inactivation of proteins and the effect of toxic O<sub>2</sub> derivatives. Enzymes can be inactivated when sensitive groups like sulphydryls are oxidized. A notable example is the nitrogen-fixation enzyme nitrogenase, which is very oxygen sensitive. [Synthesis of amino acids: Nitrogen assimilation \(section 10.5\)](#)

Oxygen accepts electrons and is readily reduced because its two outer orbital electrons are unpaired. Flavoproteins, which function in electron transport, several other cell constituents, and radiation promote oxygen reduction. The result is usually some combination of the reduction products **superoxide radical**, **hydrogen peroxide**, and **hydroxyl radical**.



These products of oxygen reduction are extremely toxic because they oxidize and rapidly destroy cellular constituents. A microorganism must be able to protect itself against such oxygen products or it will be killed. Indeed, neutrophils and macrophages, two important immune system cells, use these toxic oxygen products to destroy invading pathogens. [Phagocytosis \(section 31.3\)](#)

Many microorganisms possess enzymes that afford protection against toxic O<sub>2</sub> products (figure 6.22). 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.



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<sub>2</sub>.

Because aerobes need O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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



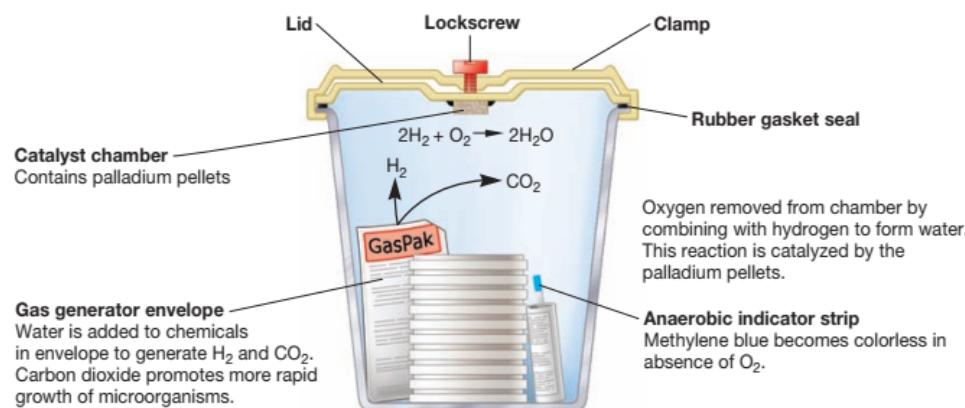
**Figure 6.23 An Anaerobic Work Chamber and Incubator.** This anaerobic system contains an oxygen-free work area and an incubator. The interchange compartment on the right of the work area allows materials to be transferred inside without exposing the interior to oxygen. The anaerobic atmosphere is maintained 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.

flushing out residual O<sub>2</sub> with nitrogen gas (figure 6.23). Often CO<sub>2</sub> as well as nitrogen is added to the chamber since many anaerobes require a small amount of CO<sub>2</sub> for best growth. (3) One of the most popular ways of culturing small numbers of anaerobes is by use of a GasPak jar (figure 6.24). In this procedure the environment is made anoxic by using hydrogen and a palladium catalyst to remove O<sub>2</sub> 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 anoxic, 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.

1. Describe the five types of O<sub>2</sub> relationships seen in microorganisms.
2. How do chemotrophic aerobes use O<sub>2</sub>?
3. What are the toxic effects of O<sub>2</sub>? How do aerobes and other oxygen-tolerant microbes protect themselves from these effects?
4. Describe four ways in which anaerobes may be cultured.

### Pressure

Organisms that spend their lives on land or on the surface of water are always subjected to a pressure of 1 atmosphere (atm), and are never affected significantly by pressure. Yet many prokaryotes live in the deep sea (ocean of 1,000 m or more in depth)



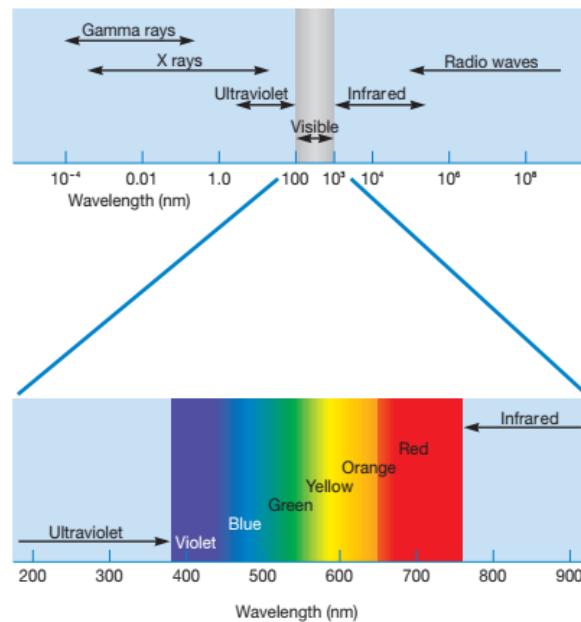
**Figure 6.24 The GasPak Anaerobic System.** Hydrogen and carbon dioxide are generated by a GasPak envelope. The palladium catalyst in the chamber lid catalyzes the formation of water from hydrogen and oxygen, thereby removing oxygen from the sealed chamber.

where the hydrostatic pressure can reach 600 to 1,100 atm and the temperature is about 2 to 3°C. Many of these prokaryotes are **barotolerant**: increased pressure adversely affects them but not as much as it does nontolerant microbes. Some prokaryotes in the gut of deep-sea invertebrates such as amphipods (shrimplike crustaceans) and holothurians (sea cucumbers) are truly **barophilic**—they grow more rapidly at high pressures. These microbes may play an important role in nutrient recycling in the deep sea. A barophile recovered from the Mariana trench near the Philippines (depth about 10,500 m) 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 archaea are thermobarophiles (e.g., *Pyrococcus* spp., *Methanocaldococcus janaschii*). [Microorganisms in marine environments \(section 28.3\)](#)

### Radiation

Our world is bombarded with electromagnetic radiation of various types (**figure 6.25**). 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: most 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



**Figure 6.25 The Electromagnetic Spectrum.** A portion of the spectrum is expanded at the bottom of the figure.

wavelengths shorter than 287 nm is absorbed by O<sub>2</sub> 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<sub>2</sub>. The fairly even distribution of sunlight throughout the visible spectrum accounts for the fact that sunlight is generally "white." [Phototrophy \(section 9.12\)](#)

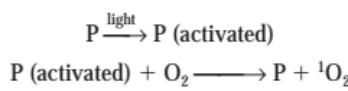
Many forms of electromagnetic radiation are very harmful to microorganisms. This is particularly true of **ionizing radiation**, radiation of very short wavelength and high energy, which can cause atoms to lose electrons (ionize). Two major forms of ionizing

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 prokaryotes (e.g., *Deinococcus radiodurans*) and bacterial endospores can survive large doses of ionizing radiation. [The use of physical methods in control: Radiation \(section 7.4\); Deinococcus-Thermus \(section 21.2\)](#)

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 ( $\text{OH}^-$ ). 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** can kill 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. Two adjacent thymines in a DNA strand are covalently joined to inhibit DNA replication and function. Microbes are protected from shorter wavelengths of UV light because they are absorbed by oxygen, as described previously. The damage caused by UV light that reaches Earth's surface can be repaired by several DNA repair mechanisms, which are discussed in chapter 13. Excessive exposure to UV light outstrips the organism's ability to repair the damage and death results. Longer wavelengths of UV light (near-UV radiation; 325 to 400 nm) are not absorbed by oxygen and so reach the Earth's surface. They can also harm microorganisms because they induce the breakdown of tryptophan 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. [Mutations and their chemical basis \(section 13.1\)](#)

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  $\text{O}_2$  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  $\text{O}_2$  generating **singlet oxygen** ( ${}^1\text{O}_2$ ).



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. [Phagocytosis \(section 31.3\)](#)

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.

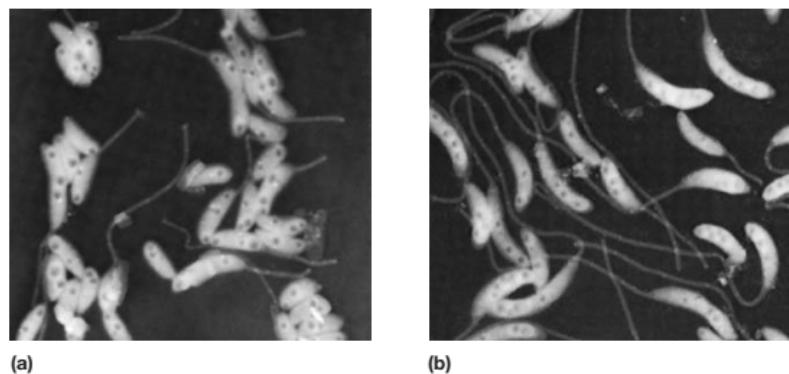
1. What are barotolerant and barophilic bacteria? Where would you expect to find them?
2. List the types of electromagnetic radiation in the order of decreasing energy or increasing wavelength.
3. Why is it so important that the Earth receives an adequate supply of sunlight? What is the importance of 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?

## 6.6 MICROBIAL GROWTH IN NATURAL ENVIRONMENTS

Section 6.5 surveys the effects on microbial growth of individual environmental factors such as water availability, pH, and temperature. Although microbial ecology is introduced in more detail in chapters 27 to 30, we now briefly consider the effect of the environment as a whole on microbial growth.

### Growth Limitation by Environmental Factors

The microbial environment is complex and constantly changing. It often contains low nutrient concentrations (**oligotrophic environment**) and exposes microbes to many overlapping gradients of nutrients and other environmental factors. The growth of microorganisms depends on both the nutrient supply and their tolerance of the environmental conditions present in their habitat at any particular time. Two laws clarify this dependence. **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 6.7) 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. **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 as shown in figure 6.21. 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. Inhibitory substances in the environment can also limit microbial growth. For instance, rapid, unlimited growth ensues if a microorganism is exposed to excess nutrients. Such growth quickly depletes nutrients and often results in the release of toxic products. Both nutrient depletion and the toxic products limit further growth. Another example is seen with microbes growing in nutrient-poor or oligotrophic environments, where the growth of microbes can be directly inhibited by a variety of natural substances including phenolics, tannins, ammonia, ethylene, and volatile sulfur compounds.



**Figure 6.26 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 phosphorous is plentiful. (b) The stalks are extremely long under phosphorus-limited conditions.

In response to 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 prokaryotes to "mini" and "ultramicro" cells or changes in the morphology of prosthecate or stalked bacteria, in response to starvation. Nutrient deprivation induces many other changes as discussed previously (figure 6.26). 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 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.

#### Counting and Identifying Microorganisms in Natural Environments

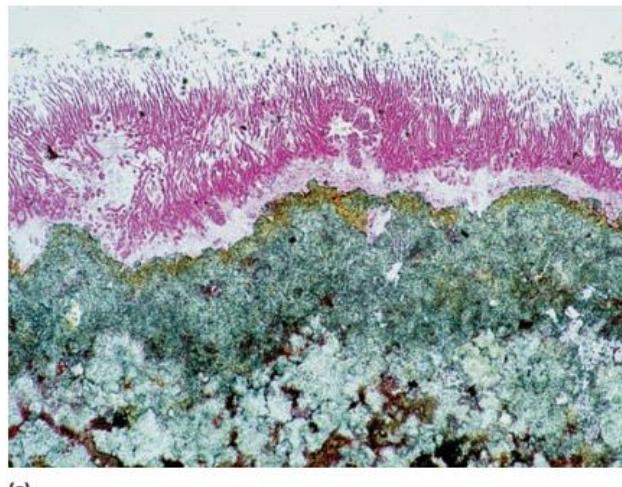
Microbial ecologists ask two important questions: What microbes are in a microbial habitat, and how many there are? Although microbiologists have developed numerous techniques for identifying and counting microbes, these questions are not easily answered. There are two reasons for this. First, many identification and counting methods rely on the ability of a microbe to form colonies. This presupposes that the microbiologist knows how to construct a growth medium and create environmental conditions that will support all the microbes in a habitat. Unfortunately, this knowledge eludes microbiologists, and it is estimated that only about 1% of the microbes in natural environments have been cultured. Increasingly, molecular methods are being used to analyze the diversity of microbial populations. The second reason is related to the "stress"

microbes experience in natural environments. John 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 that have lost 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."

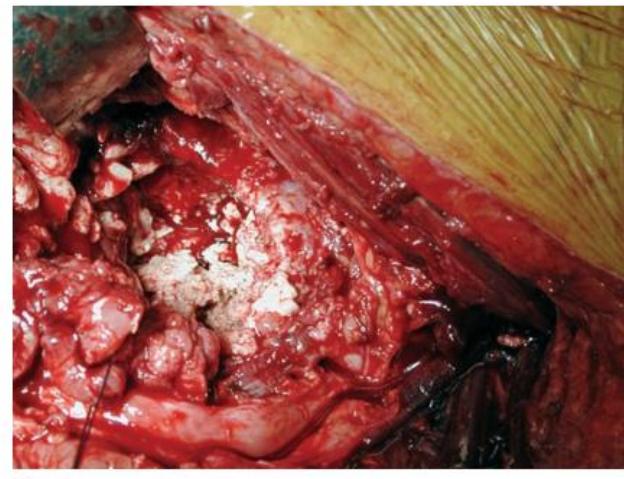
Since that time many workers have developed additional sensitive microscopic, isotopic, and molecular genetic procedures to evaluate the presence and significance of these viable but nonculturable prokaryotes (VBNC) in both lab and field. The new field of environmental genomics, or metagenomics, is discussed in chapter 15. In a more routine approach, levels of fluorescent antibody and acridine orange-stained cells often are compared with population counts obtained by the most probable number (MPN) method 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 pathogenic 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. [Microbial ecology and its methods: An overview \(section 27.4\)](#); [Water purification and sanitary analysis \(section 41.1\)](#)

#### Biofilms

Although scientists observed as early as the 1940s that more microbes in aquatic environments were found attached to surfaces (sessile) rather than were free-floating (planktonic), only relatively recently has this fact gained the attention of microbiologists. These attached microbes are members of complex, slime-encased communities called **biofilms**. Biofilms are ubiquitous in nature. There they are most often seen as layers of slime on rocks or other objects in water (figure 6.27a). When they form on the hulls of



(a)



(b)

**Figure 6.27 Examples of Biofilms.** Biofilms form on almost any surface exposed to microorganisms. (a) Biofilm on the surface of a stromatolite in Walker Lake (Nevada, USA), an alkaline lake. The biofilm consists primarily of the cyanobacterium *Calothrix*. (b) Photograph taken during surgery to remove a biofilm-coated artificial joint. The white material is composed of pus, bacterial and fungal cells, and the patient's white blood cells.

boats and ships, they cause corrosion, which limits the life of the ships and results in economic losses. Of major concern is the formation of biofilms on medical devices such as hip and knee implants (figure 6.27b). These biofilms often cause serious illness and failure of the medical device. Biofilm formation is apparently an ancient ability among the prokaryotes, as evidence for biofilms can be found in the fossil record from about 3.4 billion years ago.

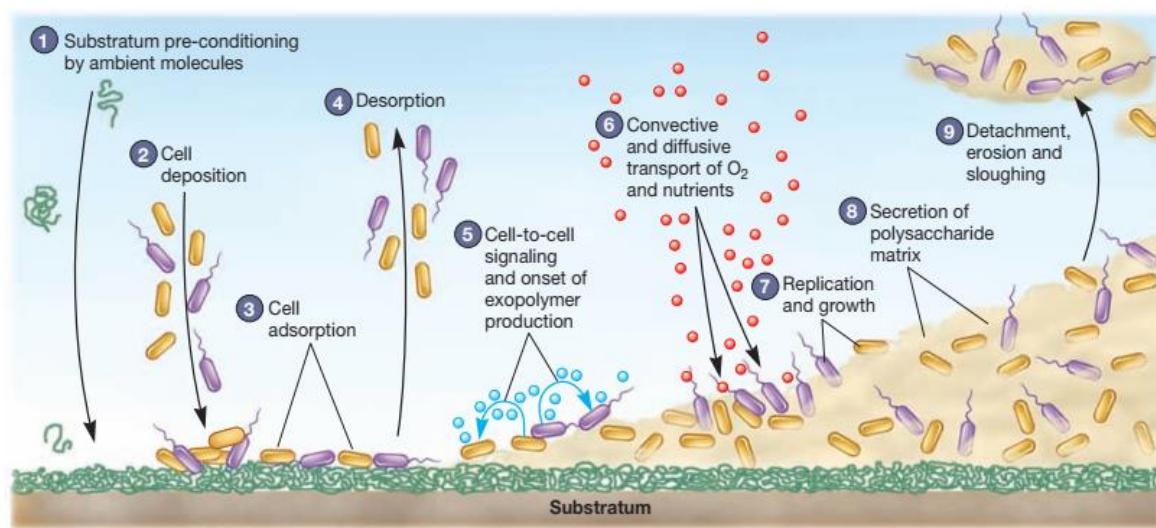
Biofilms can form on virtually any surface, once it has been conditioned by proteins and other molecules present in the environment (figure 6.28). Microbes reversibly attach to the conditioned surface and eventually begin releasing polysaccharides, proteins, and DNA. These polymers allow the microbes to stick more stably to the surface. As the biofilm thickens and matures, the microbes reproduce and secrete additional polymers. The end result is a complex, dynamic community of microorganisms. The microbes interact in a variety of ways. For instance, the waste products of one microbe may be the energy source for another microbe. The cells also communicate with each other as described next. Finally, the presence of DNA in the extracellular slime can be taken up by members of the biofilm community. Thus genes can be transferred from one cell (or species) to another.

While in the biofilm, microbes are protected from numerous harmful agents such as UV light, antibiotics, and other antimicrobial agents. This is due in part to the extracellular matrix in which they are embedded, but it also is due to physiological changes. Indeed, numerous proteins synthesized or activated in biofilm cells are not observed in planktonic cells and vice versa. The resistance of biofilm cells to antimicrobial agents has serious consequences. When biofilms form on a medical device such as a hip implant (figure 6.27b), they are difficult to kill and can cause serious illness. Often the only way to treat patients in this

situation is by removing the implant. Another problem with biofilms is that cells are regularly sloughed off (figure 6.28). This can have many consequences. For instance, biofilms in a city's water distribution pipes can serve as a source of contamination after the water leaves a water treatment facility.

#### Cell-Cell Communication Within Microbial Populations

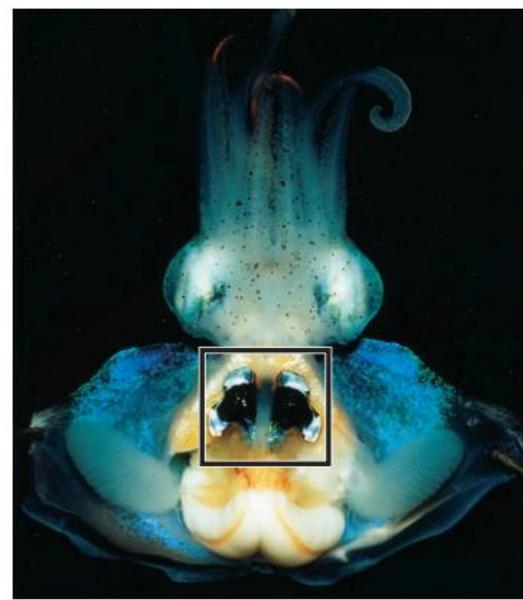
For decades, microbiologists tended to think of bacterial populations as collections of individual cells growing and behaving independently. But about 30 years ago, it was discovered that the marine luminescent bacterium *Vibrio fischeri* controls its ability to glow by producing a small, diffusible substance called autoinducer. The autoinducer molecule was later identified as an **acylhomoserine lactone (AHL)**. It is now known that many gram-negative bacteria make AHL molecular signals that vary in length and substitution at the third position of the acyl side chain (figure 6.29). In many of these species, the AHL is freely diffusible across the plasma membrane. Thus at a low cell density it diffuses out of the cell. However, when the cell population increases and AHL accumulates outside the cell, the diffusion gradient is reversed so that the AHL enters the cell. Because the influx of AHL is cell-density-dependent, it enables individual cells to assess population density. This is referred to as **quorum sensing**; a quorum usually refers to the minimum number of members in an organization, such as a legislative body, needed to conduct business. When AHL reaches a threshold level inside the cell, it serves to induce the expression of target genes that regulate a number of functions, depending on the microbe. These functions are most effective only if a large number of microbes are present. For instance, the light produced by one cell



**Figure 6.28** Biofilm Formation.

Signal and Structure	Representative Organism	Function Regulated
N-acyl homoserine lactone (AHL)	<i>Vibrio fischeri</i> <i>Agrobacterium tumefaciens</i> <i>Erwinia carotovora</i> <i>Pseudomonas aeruginosa</i> <i>Burkholderia cepacia</i>	Bioluminescence Plasmid transfer Virulence and antibiotic production Virulence and biofilm formation Virulence
Furanosylborate (AI-2)	<i>Vibrio harveyi</i>	Virulence
Cyclic thiolactone (AIP-II)	<i>Staphylococcus aureus</i>	Virulence
Hydroxy-palmitic acid methyl ester (PAME)	<i>Ralstonia solanacearum</i>	Virulence
Methyl dodecenonic acid (DSF)	<i>Xanthomonas campestris</i>	Virulence
Farnesoic acid	<i>Candida albicans</i>	Dimorphic transition and virulence

**Figure 6.29** Representative Cell-Cell Communication Molecules.

(a) *E. scolopes*, the bobtail squid

(b) Light organ

**Figure 6.30 *Euprymna scolopes*.** (a) *E. scolopes* is a warm-water squid that remains buried in sand during the day and feeds at night. (b) When feeding it uses its light organ (boxed, located on its ventral surface) to provide camouflage by projecting light downward. Thus the outline of the squid appears as bright as the water's surface to potential predators looking up through the water column. The light organ is colonized by a large number of *Vibrio fischeri* so autoinducer accumulates to a threshold concentration, triggering light production.

is not visible, but cell densities within the light organ of marine fish and squid reach  $10^{10}$  cells per milliliter. This provides the animal with a flashlight effect while the microbes have a safe and nutrient-enriched habitat (figure 6.30). In fact, many of the processes regulated by quorum sensing involve host-microbe interactions such as symbioses and pathogenicity. [Global regulatory systems: Quorum sensing \(section 12.5\)](#)

Many different bacteria use AHL signals. In addition to *V. fischeri* bioluminescence, the opportunistic pathogens *Burkholderia cepacia* and *Pseudomonas aeruginosa* use AHLs to regulate the expression of virulence factors (figure 6.29). These gram-negative bacteria cause debilitating pneumonia in people who are immunocompromised, and are important pathogens in cystic fibrosis patients. The plant pathogens *Agrobacterium tumefaciens* will not infect a host plant and *Erwinia carotovora* will not produce antibiotics without AHL signaling. Finally, *B. cepacia*, *P. aeruginosa*, as well as *Vibrio cholerae* use AHL intercellular communication to control biofilm formation, an important strategy to evade the host's immune system.

The discovery of additional molecular signals made by a variety of microbes underscores the importance of **cell-cell communication** in regulating prokaryotic processes. For instance, while only gram-negative bacteria are known to make AHLs, both gram-negative and gram-positive bacteria make autoinducer-2 (AI-2). Gram-positive bacteria usually exchange short peptides called oligopeptides instead of autoinducer-like molecules. Examples include *Enterococcus faecalis*, whose oligopeptide signal is used to

determine the best time to conjugate (transfer genes). Oligopeptide communication by *Staphylococcus aureus* and *Bacillus subtilis* is used to trigger the uptake of DNA from the environment. The soil microbe *Streptomyces griseus* produces a gamma-butyrolactone known as A-factor. This small molecule regulates both morphological differentiation and the production of the antibiotic streptomycin. Eucaryotic microbes also rely on cell-cell communication to coordinate key activities within a population. For example, the pathogenic fungus *Candida albicans* secretes farnesol acid to govern morphology and virulence.

These examples of cell-cell communication demonstrate 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 and fruiting body formation in the myxobacteria. [Isolation of pure cultures: Microbial growth on agar surfaces \(section 5.8\); Class Deltaproteobacteria: Order Myxococcales \(section 22.4\)](#)

1. How are Liebig's law of the minimum and Shelford's law of tolerance related? Why are generation times in nature usually much longer than in culture?
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 a biofilm? Why might life in a biofilm be advantageous for microbes?
5. What is quorum sensing? Describe how it occurs and briefly discuss its importance to microorganisms.

## Summary

Growth is an increase in cellular constituents and results in an increase in cell size, cell number, or both.

### 6.1 The Prokaryotic Cell Cycle

- a. Most prokaryotes reproduce by binary fission, a process in which the cell elongates and the chromosome is replicated and segregates to opposite poles of the cell prior to the formation of a septum, which divides the cell into two progeny cells (**figures 6.1** and **6.3**).
- b. Two overlapping pathways function during the prokaryotic cell cycle: the pathway for chromosome replication and segregation and the pathway for septum formation (**figure 6.2**). Both are complex and poorly understood. The partitioning of the progeny chromosomes may involve homologues of eukaryotic cytoskeletal proteins.
- c. In rapidly dividing cells, initiation of DNA synthesis may occur before the previous round of synthesis is completed. This allows the cells to shorten the time needed for completing the cell cycle.

### 6.2 The Growth Curve

- a. 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 6.6**).
- b. In the exponential phase, the population number of cells undergoing binary fission doubles at a constant interval called the doubling or generation time (**figure 6.10**). The mean growth rate constant ( $k$ ) is the reciprocal of the generation time.
- c. 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.

### 6.3 Measurement of Microbial Growth

- a. 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 (**figures 6.12** and **6.14**).
- b. 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 (**figure 6.15**).

### 6.4 The Continuous Culture of Microorganisms

- a. Microorganisms can be grown in an open system in which nutrients are constantly provided and wastes removed.
- b. 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 (**figure 6.16**).

### 6.5 The Influence of Environmental Factors on Growth

- a. Most bacteria, photosynthetic protists, 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 ( $a_w$ ).

- b. 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 (**figure 6.18** and **table 6.3**).
- c. Each species of microorganism has an optimum pH for growth and can be classified as an acidophile, neutrophile, or alkophile (**figure 6.19**).
- d. Microorganisms can alter the pH of their surroundings, and most culture media must be buffered to stabilize the pH.
- e. 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 (**figure 6.20**).
- f. 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 6.21** and **table 6.3**).
- g. 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 6.22** and **table 6.3**).
- h. 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.
- i. Most deep-sea microorganisms are barotolerant, but some are barophilic and require high pressure for optimal growth.
- j. 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. Ultraviolet (UV) radiation induces the formation of thymine dimers and strand breaks in DNA.
- k. Visible light can provide energy for the formation of reactive singlet oxygen, which will destroy cells.

### 6.6 Microbial Growth in Natural Environments

- a. 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.
- b. Many microbes form biofilms, aggregations of microbes growing on surfaces and held together by extracellular polysaccharides (**figure 6.28**). Life in a biofilm has several advantages, including protection from harmful agents.
- c. 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 (**figures 6.29** and **6.30**).

## Key Terms

acidophile 134	colony forming units (CFU) 130	lag phase 123	replisome 120
acylhomoserine lactone (AHL) 144	compatible solutes 132	log phase 123	septation 121
aerobe 139	continuous culture system 131	mean generation time 126	singlet oxygen 142
aerotolerant anaerobe 139	cytokinesis 121	mean growth rate constant ( $k$ ) 126	starvation proteins 124
alkalophile 134	doubling time 126	mesophile 138	stationary phase 124
anaerobe 139	exponential phase 123	microaerophile 139	strict anaerobe 139
balanced growth 123	extremophiles 132	MreB protein 120	superoxide dismutase (SOD) 140
barophilic 141	facultative anaerobe 139	neutrophile 134	superoxide radical 140
barotolerant 141	facultative psychrophiles 138	obligate aerobe 139	terminus 120
batch culture 123	FtsZ protein 122	obligate anaerobe 139	thermophile 138
binary fission 119	generation time 126	oligotrophic environment 142	turbidostat 132
biofilm 143	growth 119	origin of replication 120	ultraviolet (UV) radiation 142
cardinal temperatures 136	halophile 133	osmotolerant 134	unbalanced growth 123
catalase 140	hydrogen peroxide 140	programmed cell death 125	viable but nonculturable (VBNC) 125
cell cycle 119	hydroxyl radical 140	psychrophile 137	water activity ( $a_w$ ) 134
chemostat 131	hyperthermophile 139	psychrotroph 138	Z ring 122
coenocytic 119	ionizing radiation 141	quorum sensing 144	

## Critical Thinking Questions

- As an alternative to diffusible signals, suggest another mechanism by which bacteria can quorum sense.
- 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*?
- Design an experiment to determine if a slow-growing microbial culture is just exiting lag phase or is in exponential phase.
- Why do you think the cardinal temperatures of some microbes change depending on other environmental conditions (e.g., pH)? Suggest one specific mechanism underlying such change.

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