PHYSICAL CONDITIONS REQUIRED FOR GROWTH

In addition to knowing the proper nutrients for the cultivation of bacteria, it is also necessary to know the physical environment in which the organisms will grow best. Just as bacteria vary greatly in their nutritional requirements, so do they exhibit diverse responses to physical conditions such as temperature, gaseous conditions, and pH.

Temperature

Since all processes of growth are dependent on chemical reactions and since the rates of these reactions are influenced by temperature, the pattern of bacterial growth can be profoundly influenced by this condition. The temperature that allows for most rapid growth during a short period of time (12 to 24 h) is known as the optimum growth temperature. (It should be noted, however, that the optimum growth temperature thus defined may not necessarily be optimum for other cellular activities.)

Table 6-8 shows the optimum temperture for several bacteria and also the range of temperatures within which they will grow. It can be seen that the maximum temperature at which growth occurs is usually quite close to the optimum temperature, whereas the minimum temperature for growth is usually much lower than the optimum. On the basis of their temperature relationships, bacteria are divided into three main groups:

1. Psychrophiles are able to grow at 0°C or lower, though they grow best at higher temperatures. Many microbiologists restrict the term *psychrophile* to organisms that can grow at 0°C but have an optimum temperature of 15°C or lower and a maximum temperature of about 20°C; the term *psychrotroph* or *facultative psychrophile* is used for those organisms able to grow at 0°C but which grow best at temperatures in the range of about 20 to 30°C (e.g., see Fig. 6-1).

   During isolation of strict psychrophiles it is usually necessary to maintain the source samples (for example, Antarctic soil samples) at cold temperatures from the time they are collected and also to chill all media before attempting isolation. This is because strict psychrophiles usually die if they are even temporarily exposed to room temperature. Even at optimum growth temperatures, it often takes two or three weeks for colonies of psychrophiles to develop.

   The physiological factors responsible for the low temperature maxima for strict psychrophiles are not entirely clear, but some factors that have been implicated are heat instability of ribosomes and various enzymes, increased leakage of cell

Table 6-8. Characteristics of Several Species of Bacteria with Regard to Temperatures at Which They Grow

	Temperature of Growth, °C		
	Minimum	Optimum	Maximum
Vibrio marinus strain MP-1	-1	15	20
Vibrio psychroerythrus	0	15	19
Pseudomonas fluorescens	4	25–30	40
Staphylococcus aureus	6.5	30–37	46
Corynebacterium diphtheriae	15	37	40
Neisseria gonorrhoeae	30	35–36	38.5
Streptococcus thermophilus	20	40–45	50
Thermoactinomyces vulgaris	27–30	60	65–70
Thermus aquaticus	40	70–72	79

SOURCE: Data from R. Y. Morita, *Bacteriol. Rev.*, 39:144, 1975, and from *Bergey's Manual of Determinative Bacteriology*, 8th ed., Williams & Wilkins, Baltimore, 1974.

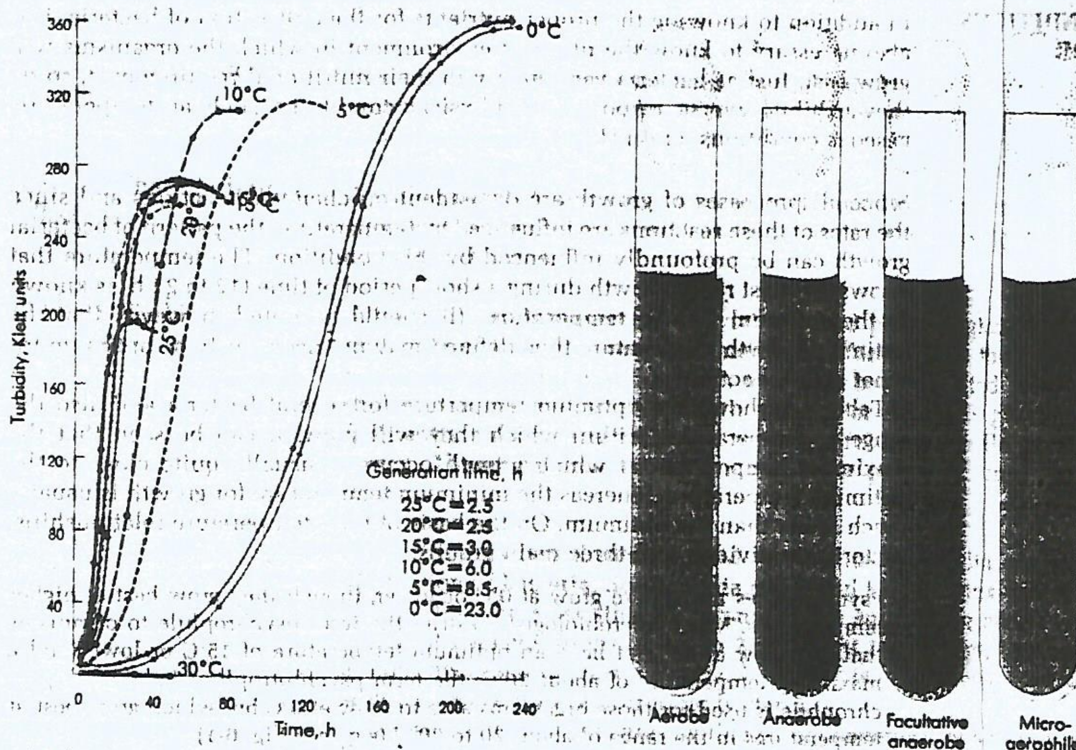


Figure 6-1. Effect of temperature on the growth of a psychrotrophic *Bacillus* sp. Note that rate of growth (measured turbidimetrically in Klett units) is more rapid at 25°C than at 0°C, although the total quantity of cells at the termination of growth is greater at the lower temperature. (*Courtesy of J. L. Stokes in Low Temperature Biology of Food Stuffs, Pergamon, New York, 1968.*)

Figure 6-2. Schematic illustration of the growth of bacteria in deep agar tubes, showing differences in response to atmospheric oxygen.

components, and impaired transport of nutrients above the maximum temperature.

2 **Mesophiles** grow best within a temperature range of approximately 25 to 40°C. For example, all bacteria that are pathogenic for humans and warm-blooded animals are mesophiles, most growing best at about body temperature (37°C).

3 **Thermophiles** grow best at temperatures above 45°C. The growth range of many thermophiles extends into the mesophilic region; these species are designated facultative thermophiles. Other thermophiles cannot grow in the mesophilic range; these are termed true thermophiles, obligate thermophiles, or stenothermophiles.

Factors that have been implicated in the ability to grow at high temperatures are an increased thermal stability of ribosomes, membranes, and various enzymes. Loss of the fluidity that exists within the lipid bilayer of the cytoplasmic membrane may be a factor governing the minimum temperature.

It is important to note that a bacterial species may not manifest the same characteristics in every detail when grown at different temperatures. For example, *Serratia marcescens* forms a blood-red to orange pigment when cultured at 25°C but produces little or no pigment when cultured at 37°C. Similarly, *Lactobacillus plantarum* does not require the amino acid phenylalanine for growth when cultured at 25°C but does require it at 37°C.

Gaseous Requirements

The principal gases that affect bacterial growth are oxygen and carbon dioxide. Bacteria display such a wide variety of responses to free oxygen that it is convenient to divide them into four groups on the following bases:

1. **Aerobic bacteria** require oxygen for growth and can grow when incubated in an air atmosphere (i.e., 21 percent oxygen).
2. **Anaerobic bacteria** do not use oxygen to obtain energy; moreover, oxygen is toxic for them and they cannot grow when incubated in an air atmosphere. Some can tolerate low levels of oxygen (**nonstringent** or **tolerant anaerobes**), but others (**stringent** or **strict anaerobes**) cannot tolerate even low levels and may die upon brief exposure to air.
3. **Facultatively anaerobic bacteria** do not require oxygen for growth, although they may use it for energy production if it is available. They are not inhibited by oxygen and usually grow as well under an air atmosphere as they do in the absence of oxygen.
4. **Microaerophilic bacteria** require low levels of oxygen for growth but cannot tolerate the level of oxygen present in an air atmosphere.

Figure 6-2 shows diagrammatically how these four classes can be distinguished by their patterns of growth in tubes in deep agar media where the diffusion of oxygen into the medium is a controlling factor.

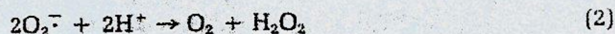
Oxygen Toxicity

Oxygen is both beneficial and poisonous to living organisms. It is beneficial because its strong oxidizing ability makes it an excellent terminal electron acceptor for the energy-yielding process known as respiration. However, oxygen is also a toxic substance. Aerobic and facultative organisms have developed protective mechanisms that greatly mitigate this toxicity, but microaerophiles and anaerobes are deficient in these mechanisms and are restricted to habitats where little or no oxygen is present. The following factors are among those that have been implicated in oxygen toxicity.

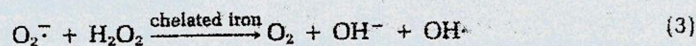
1. ***Oxygen inactivation of enzymes.*** Molecular oxygen can directly oxidize certain essential reduced groups, such as thiol (-SH) groups, or enzymes, resulting in enzyme inactivation. For instance, the enzyme complex known as nitrogenase, responsible for nitrogen fixation, is irreversibly destroyed by even small amounts of oxygen.
2. ***Damage due to toxic derivatives of oxygen.*** Various cellular enzymes catalyze chemical reactions involving molecular oxygen; some of these reactions can result in addition of a single electron to an oxygen molecule, thereby forming a **superoxide radical** ($O_2^{\cdot-}$):

$$O_2 + e^- \rightarrow O_2^{\cdot-} \quad (1)$$

Superoxide radicals can inactivate vital cell components. However, recent studies suggest that their greatest detrimental action is through production of even more toxic substances such as **hydrogen peroxide** (H_2O_2) and **hydroxyl radicals** (OH·) by means of the following reactions:



$$2O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (2)$$



$$O_2^{\cdot-} + H_2O_2 \xrightarrow{\text{chelated iron}} O_2 + OH^- + OH\cdot \quad (3)$$

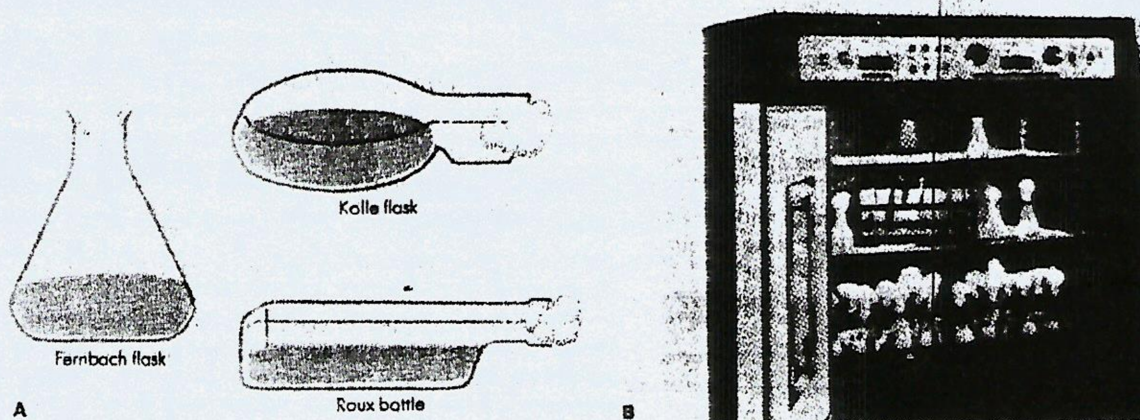
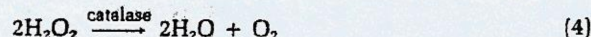


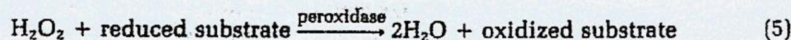
Figure 6-3. Methods for providing increased aeration during incubation. (A) Culture vessels of several designs that provide a large surface area for a shallow layer of medium. (B) An example of an incubator-shaker. The environmental chamber provides controlled conditions of temperature, humidity, and illumination. Within the chamber, flasks are fixed firmly on a platform which rotates in a circular manner, thus agitating the fluid medium constantly during incubation and exposing more culture surface to the gas phase. *(Courtesy of New Brunswick Scientific Company.)*

Hydroxyl radicals are among the most reactive free radicals known to organic chemistry and can damage almost every kind of molecule found in living cells. Hydrogen peroxide is not a free radical, but it is a powerful oxidizing agent that is highly toxic to many kinds of cells. Another toxic derivative of oxygen is an energized form known as singlet oxygen, $(^1\Delta_g)O_2$, which is produced in biological systems by certain photochemical reactions.

Aerobic and facultative organisms have developed various protective mechanisms against the toxic forms of oxygen. One is the enzyme known as superoxide dismutase, which eliminates superoxide radicals by greatly increasing the rate of reaction 2 above. The hydrogen peroxide produced by this reaction can in turn be dissipated by catalase and peroxidase enzymes:



$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \qquad (4)$$



$$H_2O_2 + \text{reduced substrate} \xrightarrow{\text{peroxidase}} 2H_2O + \text{oxidized substrate} \qquad (5)$$

Note that elimination of either superoxide radicals or hydrogen peroxide can prevent the formation of the highly dangerous hydroxyl radicals, since *both reactants* are required for reaction (3).

In general, anaerobic bacteria have either no superoxide dismutase or only relatively low levels compared to aerobes. Many anaerobes are also deficient in catalase and/or peroxidase. This may help to explain, at least in part, their sensitivity to oxygen, although other factors are probably involved as well.

Cultivation of Aerobic Bacteria. To grow aerobic or facultative bacteria in tubes or small flasks, incubation of the medium under normal atmospheric conditions is generally satisfactory. However, when aerobic organisms are to be grown in large quantities, it is advantageous to increase the exposure of the medium to the atmosphere. This can be accomplished by dispensing the medium in shallow layers, for which special containers are available. Aeration can also be increased by constantly shaking the inoculated liquid cultures (Fig. 6-3).

Cultivation of Anaerobic Bacteria. Stringent anaerobes can be grow[n by] taking special precautions to exclude all atmospheric oxygen from the me[dium]. Such an environment can be established by using one of the following metho[ds]:

1 ***Prereduced media.*** During preparation, the culture medium is boiled for several minutes to drive off most of the dissolved oxygen. A reducing agent, e.g., cysteine, is added to further lower the oxygen content. Oxygen-free N_2 is bubbled through the medium to keep it anaerobic. The medium is then dispensed into tubes which are being flushed with oxygen-free N_2, stoppered tightly, and sterilized by autoclaving. Such tubes can be stored for many months before being used. During inoculation, the tubes are continuously flushed with oxygen-free CO_2 by means of a cannula (Fig. 6-4), restoppered, and incubated.

2 ***Anaerobic chamber.*** This refers to a plastic anaerobic glove box (Fig. 6-5) that

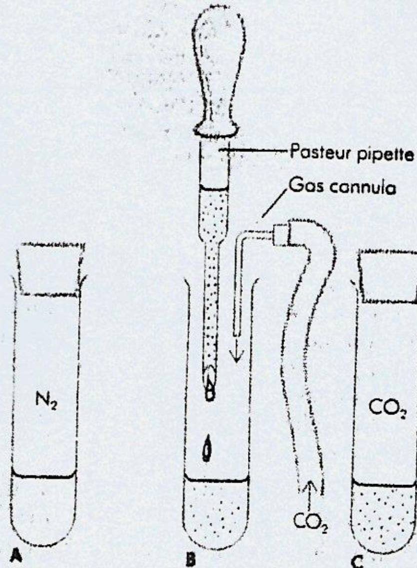
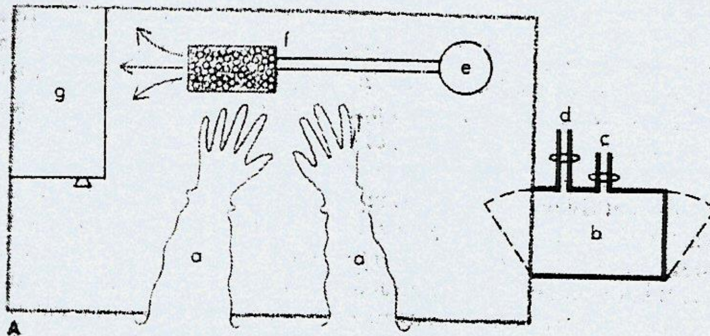


Figure 6-4. Use of prereduced media for cultivation of stringent anaerobes. (A) Tube of prereduced medium containing an atmosphere of oxygen-free N_2. (B) To inoculate, the stopper is removed and a gas cannula inserted to flush the tube continuously with oxygen-free CO_2 and maintain anaerobic conditions. The medium is inoculated with a few drops of culture by means of a Pasteur pipette. (C) After inoculation the tube is restoppered and incubated.



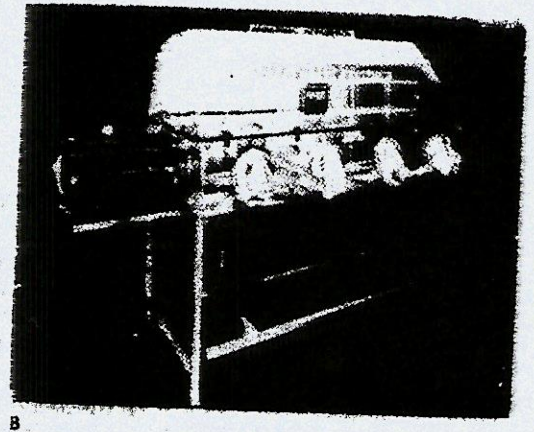


Figure 6-5. (A) Schematic diagram of the various parts of an anaerobic chamber (top view). (a) Glove ports and rubber gloves that allow the operator to perform manipulations within the chamber. (b) Air lock with inner and outer doors. Media are placed within the air lock with the inner door remaining sealed; air is removed by a vacuum pump connection (c) and replaced with N_2 through (d). The inner door is opened and the media are placed within the main chamber, which contains an atmosphere of $H_2 + CO_2 + N_2$. A circulator (e) circulates the gas atmosphere through pellets of palladium catalyst (f), causing any residual oxygen in the media to be used up by reaction with H_2. After media have become completely anaerobic they can be inoculated and placed in an incubator (g) located within the chamber. (B) Photograph of an anaerobic chamber. *(Courtesy of The Germfree Laboratories, Inc.)*

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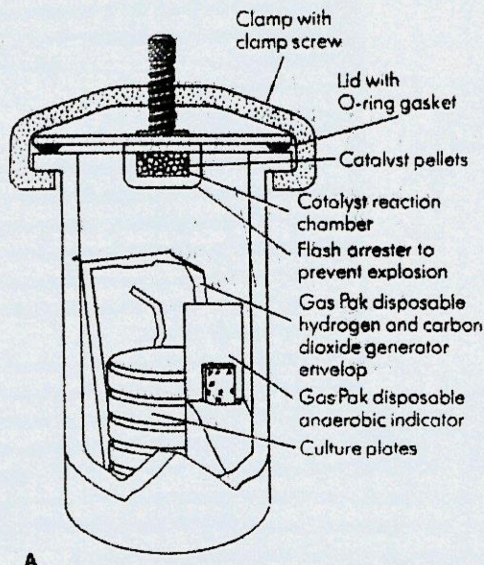

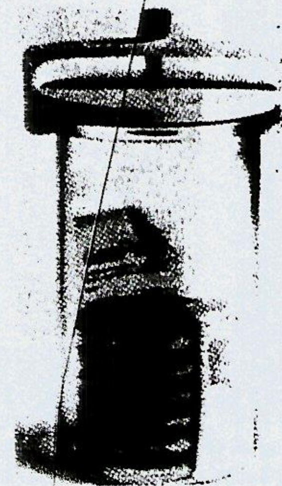

Figure 6-6. Anaerobic jar: GasPak system. (A) Media are inoculated and then placed in the jar. Water is added to the GasPak generator envelope, causing the evolution of H_2 and CO_2. The H_2 reacts with O_2 on the surface of the palladium catalyst, forming water and establishing anaerobic conditions. The CO_2 aids growth of fastidious anaerobes which sometimes fail to grow, or grow only poorly, in its absence. An anaerobic indicator strip (a pad saturated with methylene blue solution) changes from blue to colorless in the absence of oxygen. (B) The GasPak Anaerobic System with inoculated Petri dishes, the GasPak generator envelope, and the anaerobic indicator strip. *(Courtesy of BBL Microbiology Systems.)*

contains an atmosphere of H_2, CO_2, and N_2. Culture media are placed within the chamber by means of an air lock which can be evacuated and refilled with N_2. From the air lock the media are placed within the main chamber. Any O_2 in the media is slowly removed by reaction with the H_2, forming water; this reaction is aided by a palladium catalyst. After being rendered oxygen-free, the media are inoculated within the chamber (by means of the glove ports) and incubated (also within the chamber).

Nonstringent anaerobes can be cultured within an anaerobic jar such as that depicted in Fig. 6-6. Inoculated media are placed in the jar along with an H_2 + CO_2 generating system. After the jar is sealed, the oxygen present in the atmosphere within the jar, as well as that dissolved in the culture medium, is gradually used up through reaction with the hydrogen in the presence of a catalyst.

Acidity or Alkalinity (pH)

For most bacteria the optimum pH for growth lies between 6.5 and 7.5, and the limits generally lie somewhere between 5 and 9. However, a few bacteria prefer more extreme pH values for growth. For example, *Thiobacillus thiooxidans* has an optimum pH of 2.0 to 3.5 and can grow in a range between pH 0.5 and 6.0. On the other hand, an unclassified bacterium isolated from an alkaline spring in California was found to grow best at a pH of 9.0 to 9.5 and could grow within a range from 8.0 to 11.4.

When bacteria are cultivated in a medium originally adjusted to a given pH, for example, 7.0, it is very likely that this pH will change as a result of the chemical activities of the organism. If a carbohydrate is present it may be fermented or oxidized to organic acids, thus decreasing the pH of the medium. If the salt of an organic acid is supplied as a carbon source (e.g., sodium malate), its oxidation by bacteria will cause an increase in pH. Such shifts in pH may be so great that further growth of the organism is eventually inhibited.

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TABLE [illegible] **Selected Zoonoses**

Disease	Causative Agent	Reservoir	Method of Transmission	Chapter Reference
Viral				
Influenza (some types)	*Influenzavirus*	Swine, birds	Direct contact	[illegible]
Rabies	*Lyssavirus*	Bats, skunks, foxes, dogs, racoons	Direct contact (bite)	[illegible]
Western equine encephalitis	*Alphavirus*	Horses, birds	*Culex* mosquito bite	[illegible]
Hantavirus pulmonary syndrome (HPS)	*Hantavirus*	Rodents (primarily deer mice)	Direct contact with rodent saliva, feces, or urine	[illegible]
Bacterial				
Anthrax	*Bacillus anthracis*	Domestic livestock	Direct contact with contaminated hides or animals; air; food	[illegible]
Brucellosis	*Brucella* spp.	Domestic livestock	Direct contact with contaminated milk, meat, or animals	[illegible]
Bubonic plague	*Yersinia pestis*	Rodents	Flea bites	[illegible]
Cat-scratch disease	*Bartonella henselae*	Domestic cats	Direct contact	[illegible]
Ehrlichiosis	*Ehrlichia* spp.	Deer, rodents	Tick bite	[illegible]
Leptospirosis	*Leptospira*	Wild mammals, domestic dogs and cats	Direct contact with urine, soil, water	[illegible]
Lyme disease	*Borrelia burgdorferi*	Field mice	Tick bites	[illegible]
Psittacosis (ornithosis)	*Chlamydia psittaci*	Birds, especially parrots	Direct contact	24
Rocky Mountain spotted fever	*Rickettsia rickettsii*	Rodents	Tick bites	23
Salmonellosis	*Salmonella* spp.	Poultry, rats, reptiles	Ingestion of contaminated food and water and putting hands in mouth	25
Endemic typhus	*Rickettsia typhi*	Rodents	Flea bites	23
Fungal				
Ringworm	*Trichophyton* *Microsporum* *Epidermophyton*	Domestic mammals	Direct contact, fomites (nonliving objects)	21
Protozoan				
Malaria	*Plasmodium* spp.	Monkeys	Anopheles mosquito bite	23
Toxoplasmosis	*Toxoplasma gondii*	Cats and other mammals	Ingestion of contaminated meat or by direct contact with infected tissues or fecal matter	23
Helminthic				
Tapeworm (pork)	*Taenia solium*	Pigs	Ingestion of undercooked contaminated pork	25
Trichinellosis	*Trichinella spiralis*	Pigs, bears	Ingestion of undercooked contaminated pork	25

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in a very small daughter cell (termed a minicell) which lacks DNA and therefore cannot multiply.)

3 How does the completion of DNA replication initiate septum formation?

It is apparent from these and other questions that although transverse binary fission may be a primitive means of reproduction compared to that which occurs in eucaryotes, it is by no means a simple process; rather, it is the result of a precisely orchestrated series of interdependent events, many of which are not yet completely understood.

GROWTH

The most common means of bacterial reproduction is binary fission; one cell divides, producing two cells. Thus, if we start with a single bacterium, the increase in population is by geometric progression:

$$1 \rightarrow 2 \rightarrow 2^2 \rightarrow 2^3 \rightarrow 2^4 \rightarrow 2^5 \ldots 2^n$$

where n = the number of generations. Each succeeding generation, assuming no cell death, doubles the population. The total population N at the end of a given time period would be expressed

$$N = 1 \times 2^n \qquad (1)$$

However, under practical conditions, the number of bacteria N_0 inoculated at time zero is not 1 but more likely several thousand, so the formula now becomes

$$N = N_0 \times 2^n \qquad (2)$$

Solving Eq. (2) for n, we have

$$\log_{10}N = \log_{10}N_0 + n \log_{10}2$$

$$n = \frac{\log_{10} N - \log_{10}N_0}{\log_{10}2} \qquad (3)$$

If we now substitute the value of $\log_{10} 2$, which is 0.301, in the above equation, we can simplify the equation to

$$n = \frac{\log_{10}N - \log_{10}N_0}{0.301}$$

$$n = 3.3\,(\log_{10}N - \log_{10}N_0) \qquad (4)$$

Thus, by use of Eq. (4), we can calculate the number of generations that have taken place, providing we know the initial population and the population after growth has occurred.

Normal Growth Cycle (Growth Curve) of Bacteria

Assume that a single bacterium has been inoculated into a flask of liquid culture medium which is subsequently incubated. Eventually the bacterium will undergo binary fission and a period of rapid growth will ensue in which the cells multiply at an exponential rate. During this period of rapid growth, if we used the theoretical number of bacteria which should be present at various intervals of time and then plotted the data in two ways (logarithm of number of bacteria and arithmetic number of bacteria versus time), we would obtain the curve shown in Fig. 7-5. Here, the population increases regularly, doubling at regular

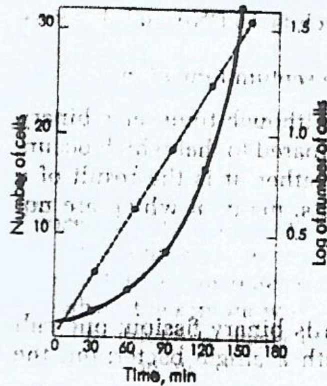


Figure 7-5. Hypothetical bacterial growth curve, assuming that one bacterial cell is inoculated into a medium and divisions occur regularly at 30-min intervals (generation time). — — — = logarithm of number of bacteria versus time; ——— = arithmetic number of bacteria versus time.

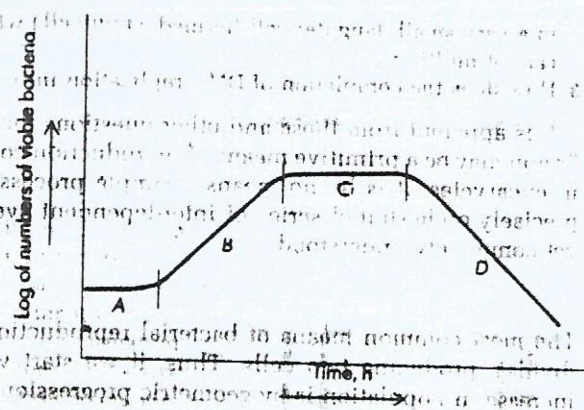


Figure 7-6. Typical bacterial growth curve. A, lag phase; B, log (logarithmic), or exponential, phase; C, stationary phase; D, death or decline phase.

time intervals (the generation time) during incubation. However, exponential growth represents only one specific portion of the growth cycle of a population. In reality, when we inoculate a fresh medium with a given number of cells, determine the bacterial population intermittently during an incubation period of 24 h (more or less), and plot the logarithms of the number of cells versus time, we obtain a curve of the type illustrated in Fig. 7-6. From this it can be seen that there is an initial period of what appears to be no growth (the lag phase), followed by rapid growth (the exponential or logarithmic phase), then a leveling off (stationary phase), and finally a decline in the viable population (death or decline phase). Between each of these phases there is a transitional period (curved portion). This represents the time required before all cells enter the new phase. Let us examine what happens to the bacterial cells during each of the phases of the growth curve.

The Lag Phase

The addition of inoculum to a new medium is not followed immediately by a doubling of the population. Instead, the population remains temporarily unchanged, as illustrated in Fig. 7-6. But this does not mean that the cells are quiescent or dormant; on the contrary, during this stage the individual cells increase in size beyond their normal dimensions. Physiologically they are very active and are synthesizing new protoplasm. The bacteria in this new environment may be deficient in enzymes or coenzymes which must first be synthesized in amounts required for optimal operation of the chemical machinery of the cell. Time for adjustments in the physical environment around each cell may be required. The organisms are metabolizing, but there is a lag in cell division.

At the end of the lag phase, each organism divides. However, since not all organisms complete the lag period simultaneously, there is a gradual increase in the population until the end of this period, when all cells are capable of dividing at regular intervals.

The Logarithmic or Exponential Phase

During this period the cells divide steadily at a constant rate, and the log of the number of cells plotted against time results in a straight line (Figs. 7-5 and 7-6). Moreover, the population is most nearly uniform in terms of chemical composition of cells, metabolic activity, and other physiological characteristics.

Table 7-1. Generation Times of Several Species of Bacteria

Bacterium	Medium	Temperature, °C	Generation Time, min
Escherichia coli	Milk	37	12.5
	Broth	37	17
Bacillus thermophilus	Broth	55	18.3
Streptococcus lactis	Milk	37	26
	Lactose broth	37	48
Staphylococcus aureus	Broth	37	27–30
Bacillus mycoides	Broth	37	28
Lactobacillus acidophilus	Milk	37	66–87
Bradyrhizobium japonicum	Mineral salts + yeast extract + mannitol	25	344–461
Mycobacterium tuberculosis	Synthetic	37	792–932
Treponema pallidum	Rabbit testes	37	1,980

SOURCE: W. S. Spector (ed.): *Handbook of Biological Data*, table 75, Saunders, Philadelphia, 1956.

The generation time g (the time required for the population to double) can be determined from the number of generations n that occur in a particular time interval t. Using Eq. (4) for n, the generation time can be calculated by the following formula:

$$g = \frac{t}{n} = \frac{t}{3.3\,(\log_{10} N - \log_{10} N_0)} \tag{5}$$

Not all bacteria have the same generation time; for some, such as *E. coli*, it may be 15 to 20 minutes; for others it may be many hours (see Table 7-1). Similarly, the generation time is not the same for a particular species under all conditions. It is strongly dependent upon the nutrients in the medium and on prevailing physical conditions, such as those outlined in Chap. 6.

During exponential growth, the growth rate (i.e., the number of generations per hour), termed R, is the reciprocal of the generation time g. It is also the slope of the straight line obtained when the log number of cells is plotted against time:

$$R = \frac{3.3(\log_{10} N - \log_{10} N_0)}{t} \tag{6}$$

You may ask how this growth rate can remain constant during the logarithmic phase of growth even though the concentration of substrate (i.e., some essential nutrient in the culture medium, usually the carbon and energy source) is continually decreasing through utilization by the organisms. To understand this, one must recognize that the relationship between R and substrate concentration is not a simple linear relationship, as shown in Fig. 7-7. When the substrate concentration is high, a change in the concentration has very little effect on the growth rate. It is only when the substrate concentration becomes quite low that the growth rate begins to decrease significantly. Since bacteria are commonly "overfed" in laboratory culture, (i.e., are supplied with far greater substrate concentrations then they need), they can multiply at a constant exponential rate for many generations before the substrate level becomes low enough to affect this rate.

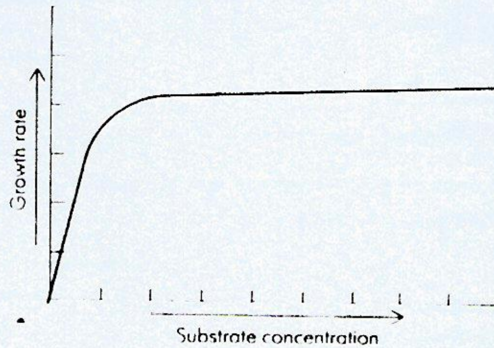


Figure 7-7. The effect of nutrient (substrate) concentration upon the growth rate of a bacterial culture. The level of substrate commonly provided in a bacterial culture is sufficiently high (right portion of curve) so that, even though the bacteria use up some substrate during the log phase of growth, the growth rate does not decrease appreciably. It is only when substrate levels become very low (left portion of curve) that the growth rate begins to be severely affected.

A microbiologist must be able to calculate growth rates and generation times. For example, it is often essential to predict how long it will take a certain population to grow to a given level. An appreciation of the full meaning of the normal growth curve is also necessary; it must be understood that during some phases of growth the cells are young and actively metabolizing while during others they are dying, so that there may be enormous structural and physiological differences between cells harvested at different times. Physical conditions and chemical substances may also affect organisms differently during different phases. Because, in general, cells in the logarithmic phase of growth are the most uniform and are in a more clearly defined condition than in any other phase, log-phase cultures are commonly used for studies of microbial metabolism.

The Stationary Phase

The logarithmic phase of growth begins to taper off after several hours, again in a gradual fashion represented by the transition from a straight line through a curve to another straight line, the stationary phase, as shown in Fig. 7-6. This trend toward cessation of growth can be attributed to a variety of circumstances, particularly the exhaustion of some nutrients, and, less often, the production of toxic products during growth. The population remains constant for a time, perhaps as a result of complete cessation of division or perhaps because the reproduction rate is balanced by an equivalent death rate.

The Phase of Decline or Death

Following the stationary phase the bacteria may die faster than new cells are produced, if indeed some cells are still reproducing. Undoubtedly a variety of conditions contribute to bacterial death, but the most important are the depletion of essential nutrients and the accumulation of inhibitory products, such as acids. During the death phase, the number of viable cells decreases exponentially, essentially the inverse of growth during the log phase. Bacteria die at different rates, just as they grow at different rates. Some species of Gram-negative cocci die very rapidly, so that there may be very few viable cells left in a culture after 72 h or less. Other species die so slowly that viable cells may persist for months or even years.

Transitional Periods Between Growth Phases

Note that a culture proceeds gradually from one phase of growth to the next (Fig. 7-6). This means that not all the cells are in an identical physiological

PETROLEUM MICROBIOLOGY

Microorganisms are associated with petroleum in its formation, its recovery by drilling, its decomposition, and its utilization. Only in recent decades has a significant amount of attention been directed to research in this field. Studies in petroleum microbiology require interdisciplinary participation. The microbiologist needs to work closely with chemists, engineers, physicists, and perhaps representatives from other fields of study. Some aspects of microbial involvement in this area are summarized as follows:

Petroleum Formation

Much of the sedimentary material of the marine environment consists of dead microbial cells. Furthermore, biochemical changes in the sedimentary deposit are accomplished by a variety of microorganisms. It is speculated that these changes are associated with the formation of petroleum.

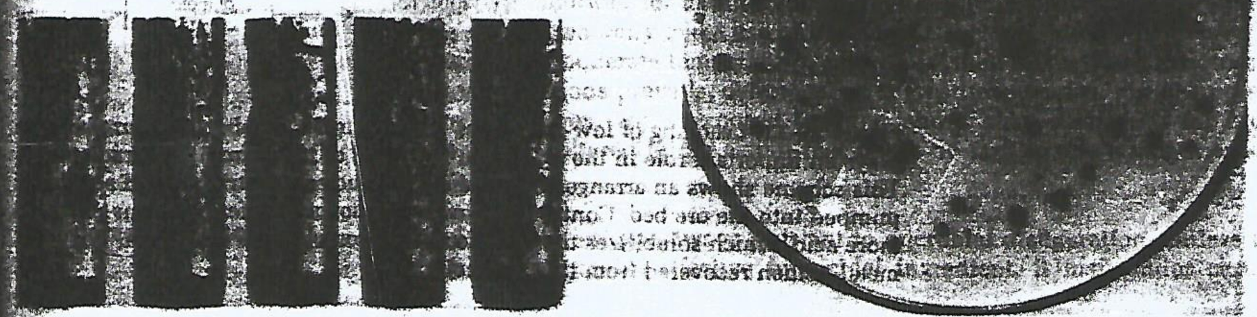
Petroleum Exploration

Soil in the region of a petroleum reservoir may contain vapors of hydrocarbon compounds such as methane or ethane. These may be detected by exposing microbial cultures in a test system which contains all nutrients for growth with the exception of a carbon source. Alternatively, the isolation of a large number of hydrocarbon-oxidizing microorganisms from soil may suggest that their presence is due to continued release of hydrocarbons from a petroleum deposit.

Petroleum Recovery

When an oil well is drilled, the initial recovery is made possible by the pressure within the rock formation. Later, as the original pressure decreases and the oil flow lessens, additional wells are drilled and water or steam is injected to force oil to the surface. Microbial activity has been suggested as a potential means of enhancing the yield of trapped oil. For example, bacteria injected into the oil might produce acid to dissolve rock formations, thereby releasing oil. Through other metabolic activities, microorganisms may decrease the viscosity of the oil.

Figure 29-10. (A) Corroded cast iron pipes from a tidal marsh. Corrosion is due primarily to activities of sulfate-reducing bacteria. (B) *Desulfovibrio* sp. growing on an iron salts-agar medium. The colonies appear black because of iron sulfide formation. *Desulfovibrio* spp. occur widely in fresh, polluted, marine, and brackish waters. (*Courtesy of W. P. Iverson and the National Bureau of Standards, U.S. Department of Commerce.*)



Corrosion of iron pipe by *Desulfovibrio* spp. is a major problem in the
industry (see Fig. 29-10). Contamination of drilling fluids by various bacteri
species is likewise a serious and costly problem.

Oil Spills

The international traffic of oil in supertankers, with the occasional acciden
that result in huge oil spills, has created a major threat to the envirenment. Ho
do we clean up the oil? One approach is to inoculate the spill area with
microorganism that has the ability to degrade petroleum oil. This concept ha
been enhanced by genetically engineering a species of *Pseudomonas putida*
that it has the capacity to metabolize the four major hydrocarbons of petroleu
camphor, octane, xylene, and naphthalene. A bacterium with this metaboli
capability made legal history by being the first genetically engineered microor
ganism ever patented.

MICROBIOLOGY AND MINING

Microorganisms play a role in the recovery of minerals from ores. Their impo
tance as agents in the process of extracting metals from ores is likely to incre
for the following reasons:

1 The richer mineral deposits are being depleted. Lower-quality ores are bei
processed, and they require development of techniques which yield more ne
complete extraction of metals.

2 The traditional method of processing ores, namely smelting, is a major cause
air pollution and is under attack from environmental groups.

Microorganisms are capable of improving both these situations. For examp
some autotrophic, aerobic bacteria (*Thiobacillus thiooxidans* and *Thiobacil
ferrooxidans*) when grown in the presence of copper ores produce acid an
effect oxidation of the ore with subsequent precipitation (removal) of the me
This process is known as **leaching.** This technique improves the recovery
metal from an ore and is nonpolluting to the atmosphere.

An example of a low-grade ore undergoing bacterial leaching is shown
Fig. 29-11.

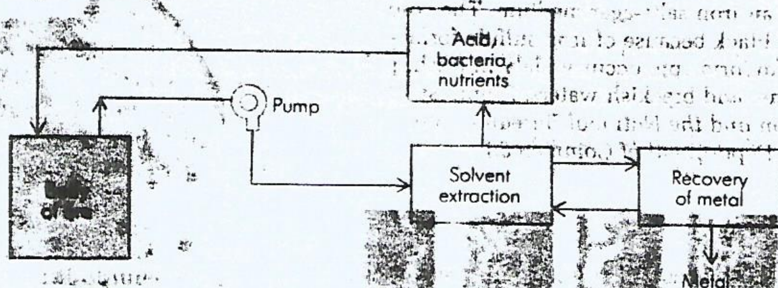


Figure 29-11. Leaching of low-grade ores using bacteria. *Thiobacillus ferrooxi*
plays an important role in the extraction (leaching) of metal from low-grade o
This scheme shows an arrangement whereby the bacteria, nutrients, and acid
pumped into the ore bed. Continued growth of *Thiobacillus ferrooxidans* pro
more acid, which solubilizes the metal content, promoting its extraction. The
metal is then recovered from this acid solution.