Microbial Growth

The requirements for microbial growth can be divided into two main categories: physical and chemical. Physical aspects include temperature, pH, and osmotic pressure. Chemical requirements include sources of carbon, nitrogen, sulfur, phosphorus, oxygen, trace elements, and organic growth factors.

Physical Requirements

Temperature

Most microorganisms grow well at the temperatures that humans favor. However, certain bacteria are capable of growing at extremes of temperature that would certainly hinder the survival of almost all eukaryotic organisms.

Microorganisms are classified into three primary groups on the basis of their preferred range of temperature: **psychrophiles** (cold-loving microbes), **mesophiles** (moderate-temperature– loving microbes), and **thermophiles** (heat-loving microbes).

Each bacterial species grows at particular **minimum**, **optimum**, and **maximum** temperatures. The minimum growth temperature is the lowest temperature at which the species will grow. The optimum growth temperature is the temperature at which the species grows best. The maximum growth temperature is the highest temperature at which growth is possible.

pH

pH refers to the acidity or alkalinity of a solution. Most bacteria grow best in a narrow pH range near neutrality, between pH 6.5 and 7.5. Very few bacteria grow at an acidic pH (acidophiles) below about pH 4.

Osmotic Pressure

Microorganisms obtain almost all their nutrients in solution from the surrounding water. Thus, they require water for growth. High osmotic pressures have the effect of removing necessary water from a cell. This osmotic loss of water causes **plasmolysis**, or **shrinkage** of the cell’s cytoplasm.

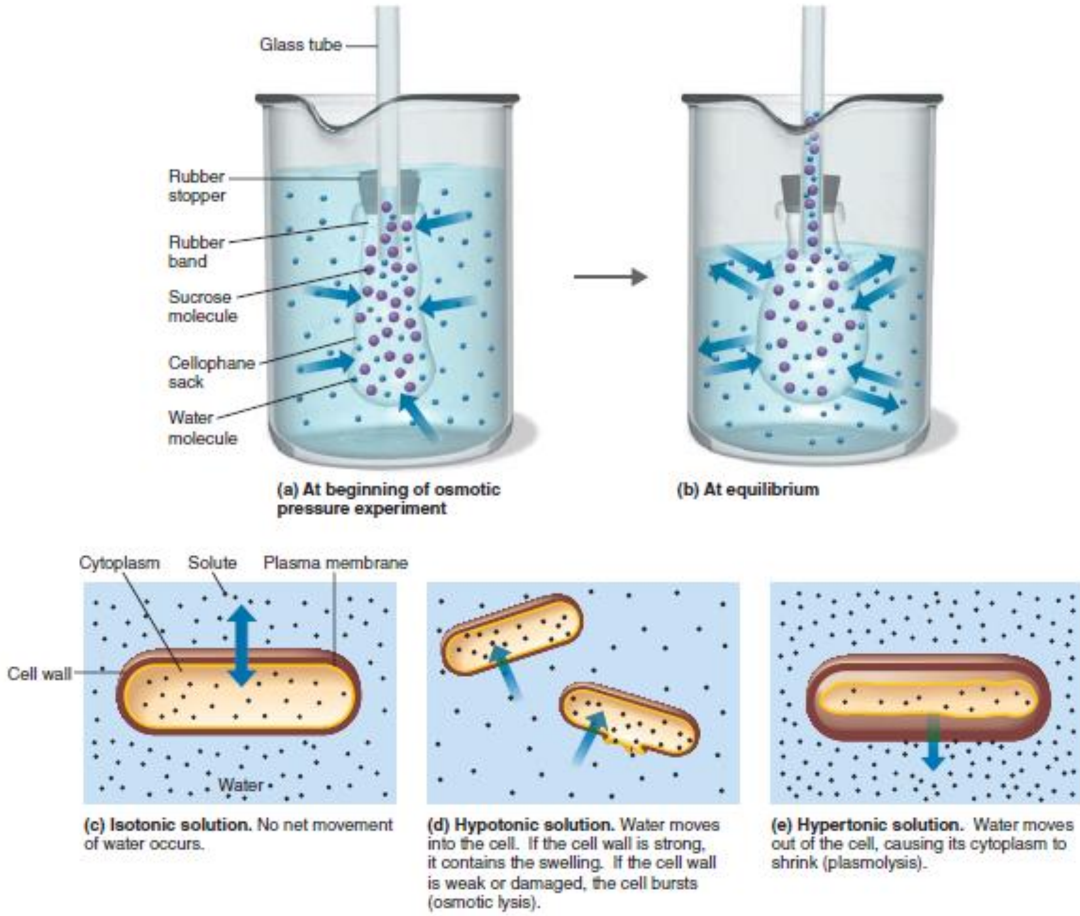
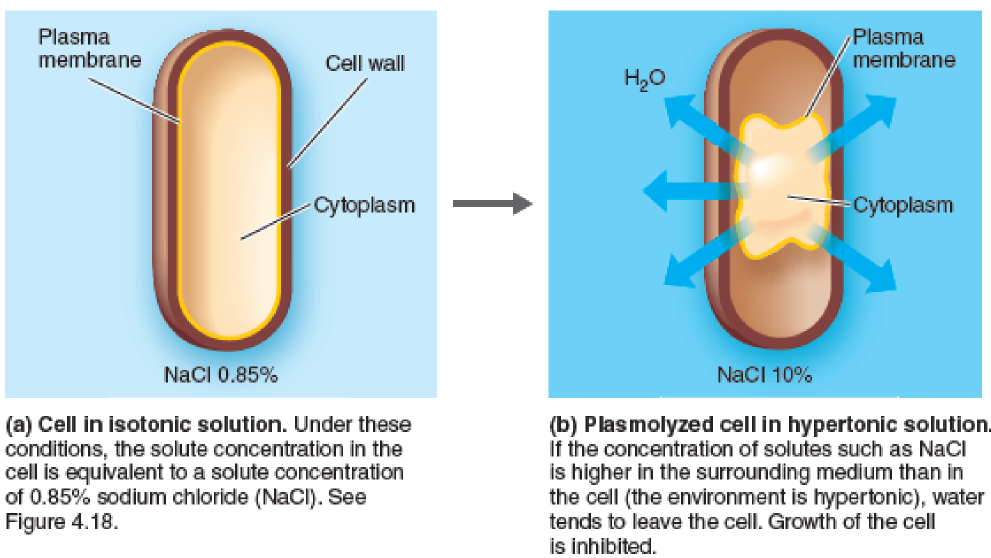


Figure 4.1 The principle of osmosis. (**a**) Setup at the beginning of an osmotic pressure experiment. Water molecules start to move from the beaker into the sack along the concentration gradient.
(**b**) Setup at equilibrium. The osmotic pressure exerted by the solution in the sack pushes water molecules from the sack back into the beaker to balance the rate of water entry into the sack. The height of the solution in the glass tube at equilibrium is a measure of the osmotic pressure. (**c**)–(**e**) The effects of various solutions on bacterial cells. (Tortora, an introduction to Microbiology, page, 92).



Plasmolysis (Tortora, an introduction to Microbiology, page, 157).

Chemical Requirements

Carbon

Besides water, one of the most important requirements for microbial growth is carbon. Carbon is the structural backbone of living matter; it is needed for all the organic compounds that make up a living cell.

Nitrogen, Sulfur, and Phosphorus

In addition to carbon, microorganisms need other elements to synthesize cellular material. For example, protein synthesis requires considerable amounts of nitrogen as well as some sulfur.

The syntheses of DNA and RNA also require nitrogen and some phosphorus, as does the synthesis of ATP, the molecule so important for the storage and transfer of chemical energy within the cell.

Sulfur is used to synthesize sulfur-containing amino acids and vitamins such as thiamine and biotin.

Phosphorus is essential for the synthesis of nucleic acids and the phospholipids of cell membranes. Among other places, it is also found in the energy bonds of ATP.

Potassium, magnesium, and calcium are also elements that microorganisms require, often as cofactors for enzymes.

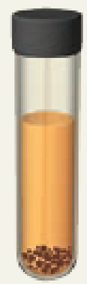
Trace Elements

Microbes require very small amounts of other mineral elements, such as iron, copper, molybdenum, and zinc; these are referred to as **trace elements.**

Oxygen

In table 4.1 the lecture summarize the effect of oxygen on the growth of various types of bacteria.

Table 4.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Culture media

A nutrient material prepared for the growth of microorganisms in a laboratory is called a culture medium. Some bacteria can grow well on just about **any culture medium**; others require **special media**, and still **others cannot grow** on any nonliving medium yet developed. Microbes that are introduced into a culture medium to initiate growth are called an **inoculum**. The microbes that grow and multiply in or on a culture medium are referred to as a **culture**.

Culture medium must contain the **right nutrients** for the specific microorganism. It should also contain sufficient **moisture**, a properly adjusted **pH**, and a suitable level of **oxygen**, perhaps none at all.

The medium must initially be **sterile**—that is, **it must initially contain no living microorganisms**—so that the culture will contain only the microbes (and their offspring) we add to the medium. Finally, the growing culture should be incubated at the **proper temperature**.

When it is desirable to grow bacteria on a solid medium, a solidifying agent such as agar is added to the medium. A complex polysaccharide derived from a marine alga, **agar**. **Agar** liquefies at about **100**°C (the boiling point of water) and at sea level remains liquid until the temperature drops to about **40**°C. For laboratory use, agar is held in water baths at about **50**°C.

Agar media are usually contained in **test tubes** or **Petri dishes**. The test tubes are called **slants** when their contents are allowed to solidify with the tube **held at an angle** so that a large surface area for growth is available. When the agar solidifies in a **vertical** tube, it is called a **deep**.

A medium in which all **chemical components** are known is a **defined** or **synthetic medium.** Media that contain some ingredients of **unknown chemical composition** are **complex media.**

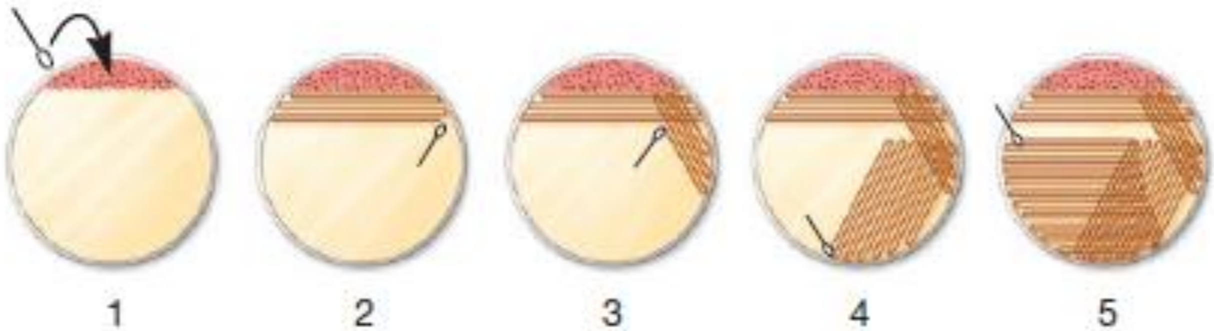
Functional Types of Media

- Media such as **tryptic soy broth** and **tryptic soy agar** are called general purpose or **supportive media**. because they sustain the growth of many microorganisms.
- Blood and other nutrients may be added to supportive media to **encourage** the growth of **fastidious** microbes. These fortifiedالمعزز media (e.g., blood agar) are called **enriched media**.
- **Selective media** allow the growth of particular microorganisms, while inhibiting the growth of others, such as **Eosin methylene blue agar** and **MacConkey agar**
- **Differential media** are media that distinguish among different groups of microbes. Blood agar is both a differential medium and an enriched one. It distinguishes between hemolytic and nonhemolytic bacteria, MacConkey agar is both differential and selective.

Cultivation of the Microorganisms

1. Streak plate technique

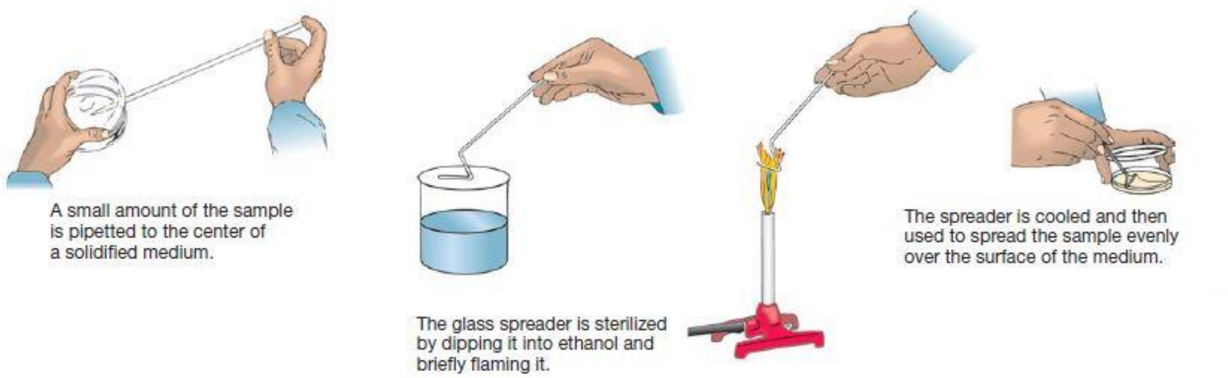
One method for separating cells is the streak plate. In this technique, cells are transferred to the edge of an agar plate with an inoculating loop or swab and then streaked across the surface in one of several patterns. After the first sector is streaked, the inoculating loop is sterilized and an inoculum for the second sector is obtained from the first sector. A similar process is followed for streaking the third sector, except that the inoculum is from the second sector.



Streak-Plate Technique. A typical streaking pattern (Prescott's Microbiology, 159)

Spread Plate and Pour Plate

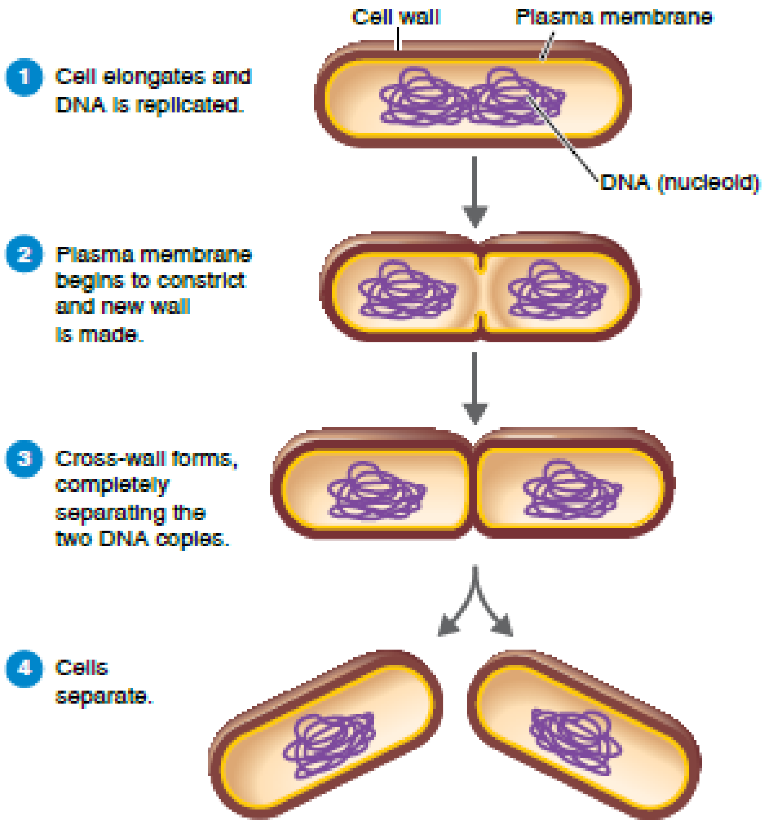
Spread-plate and **pour**-plate techniques are similar in that they both **dilute** a sample of cells before separating them spatially مكانيا. They differ in that the spread plate spreads the cells on the surface of the agar, whereas the **pour plate** embeds the cells **within** the agar.



Spread-Plate Technique. The preparation of a spread plate. (Prescott's Microbiology, 159)

Bacterial Growth Curve

Bacterial growth refers to an increase in bacterial numbers, not an increase in the size of the individual cells. Bacteria normally reproduce by binary fission.



A diagram of the sequence of cell division (Tortora, p. 165)

Bacterial population obtained after inoculation of the bacterium into a new culture medium.

The normal bacterial growth curve has four phases.

1. Lag phase

The period of **adaptation** with active macro-molecular synthesis like **DNA**, **RNA**, **various enzymes** and other structural components. It is the preparation time for reproduction; **no increase** in cell number.

2. Exponential(log) phase

The period of **active multiplication** of cells. Cell division precedes at a **logarithmic** rate, and determined by the medium and condition of the culture.

3. Maximal stationary phase

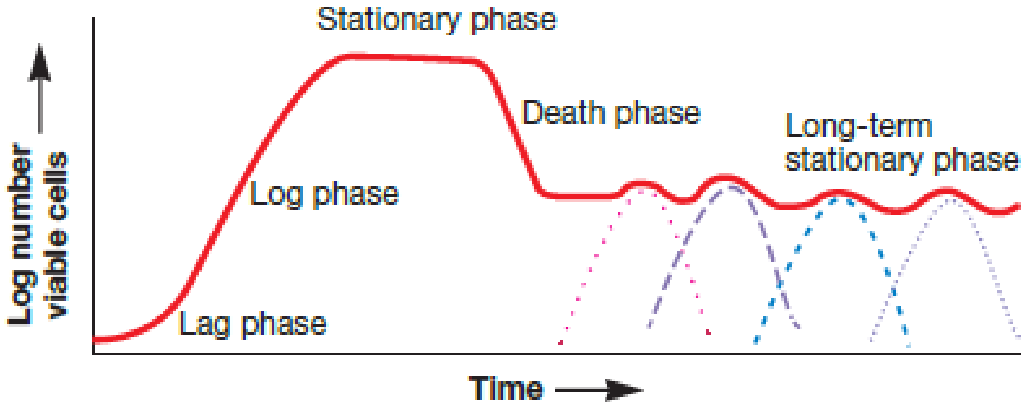
The period when the bacteria have achieved their maximal cell density or yield.

There is no further increase in viable bacterial cell number. The growth rate is exactly equal to the death rate. A bacterial population may reach stationary growth when one of the following conditions occur:

1. The required nutrients are exhausted.
2. Inhibitory end products are accumulated.
3. Physical conditions do not permit a further increase in population size.

4. Decline phase

The period at which the rate of death of bacterial cells exceeds the rate of new cell formation. There is drastic decline in viable cells. Few organisms may persist for so long time at this period at the expense of nutrients released from dying micro-organisms.



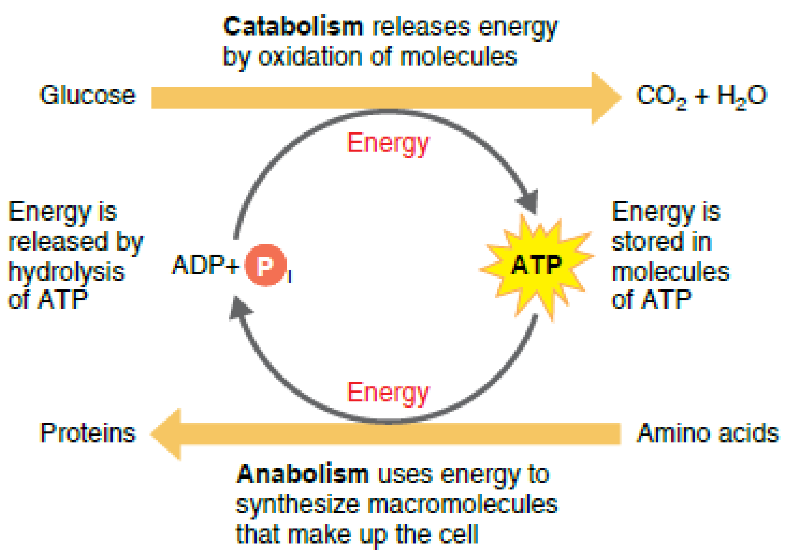
Microbial Growth Curve in a Closed System. (Prescott's, p161)

Bacterial Metabolism

The term **metabolism** refer to the sum of all chemical reactions within a living organism. Because chemical reactions either release or require energy, metabolism can be viewed as an energy-balancing act. Accordingly, metabolism can be divided into two classes of chemical reactions: those that release energy and those that require energy.

In living cells, the enzyme-regulated chemical reactions that release energy are generally the ones involved in **catabolism**, the breakdown of complex organic compounds into simpler ones. These reactions are called *catabolic*, or *degradative*, reactions. Catabolic reactions are generally hydrolytic reactions (reactions which use water and in which chemical bonds are broken), and they are exergonic (produce more energy than they consume). An example of catabolism occurs when cells break down sugars into carbon dioxide and water.

The enzyme-regulated energy-requiring reactions are mostly involved in **anabolism**, the building of complex organic molecules from simpler ones. These reactions are called *anabolic*, or *biosynthetic*, reactions. Anabolic processes often involve dehydration synthesis reactions (reactions that release water), and they are endergonic (consume more energy than they produce). Examples of anabolic processes are the formation of proteins from amino acids, nucleic acids from nucleotides, and polysaccharides from simple sugars. These biosynthetic reactions generate the materials for cell growth.



The role of ATP in coupling anabolic and catabolicreactions. (Tortora, p. 110)