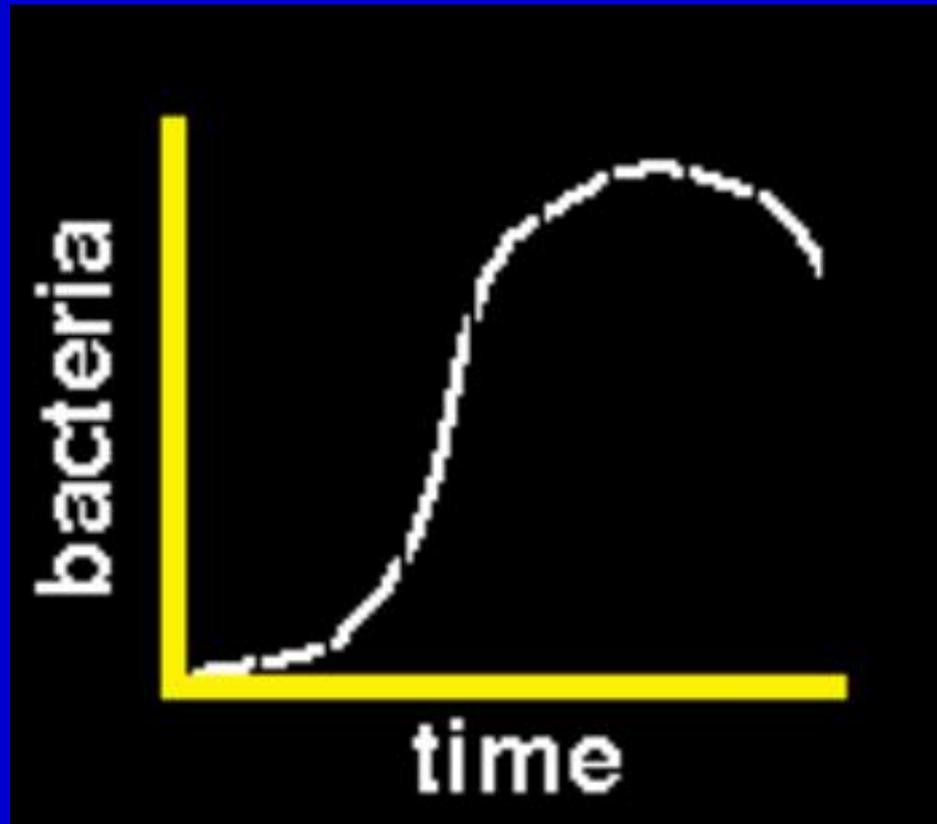


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Chapter 7

Microbial Growth and Growth control



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Chapter outline

7.1 Overview of Cell Growth

7.2 Population Growth

7.3 Measurement of Growth

7.4 Continuous Culture: The Chemostat

7.5 Effect of Environment on Growth

7.6 Growth Control

7.7 Viral Control

7.8 Fungal Control

Concepts

- Microbial growth is defined as an increase in cellular constituents and may result in an increase in a microorganism's size, population number, or both.
- 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.
- Solid objects can be sterilized by physical agents such as heat and radiation; Liquids and gases are sterilized by heat, radiation, and filtration through the proper filter.
- A knowledge of methods used for microbial control is essential for personal and public safety.

7.1 Overview of microbial growth

The bacterial cell is a synthetic machine that is able to duplicate itself. The processes involve as many as 2000 chemical reactions of a wide variety of types:

- Energy transformations.
- Biosynthesis of small molecules--the building blocks of macromolecules--as well as the various cofactors and coenzymes needed for enzymatic reactions.

Growth definition

Growth may be generally defined as a steady increase in all of the chemical components of an organism. Growth usually results in an increase in the size of a cell and frequently results in cell division.

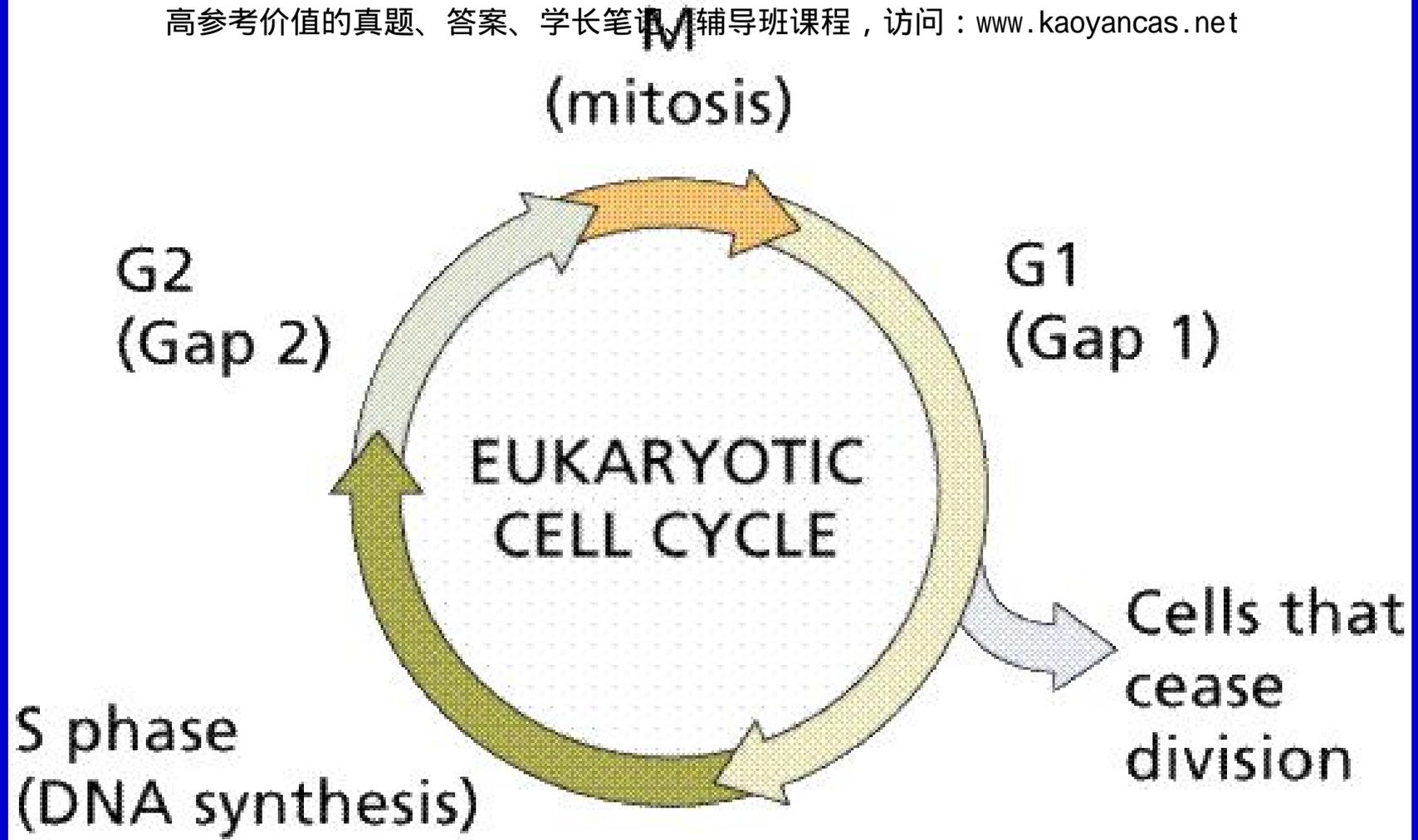
Cell life cycle in Eukaryotic cells

G₁ Primary growth phase of the cell during which cell enlargement occurs, a gap phase separating cell growth from replication of the genome

S Phase in which replication of the genome occurs

G₂ Phase in which the cell prepares for separation of the replicated genomes, this phase includes synthesis of microtubules and condensation of DNA to form coherent chromosomes, a gap phase separating chromosome replication from miosis.

M Phase called miosis during which the microtubular apparatus is associated and subsequently used to pull apart the sister chromosomes.



Eukaryotic cell: $G1 \longrightarrow S \longrightarrow G2 \longrightarrow M$

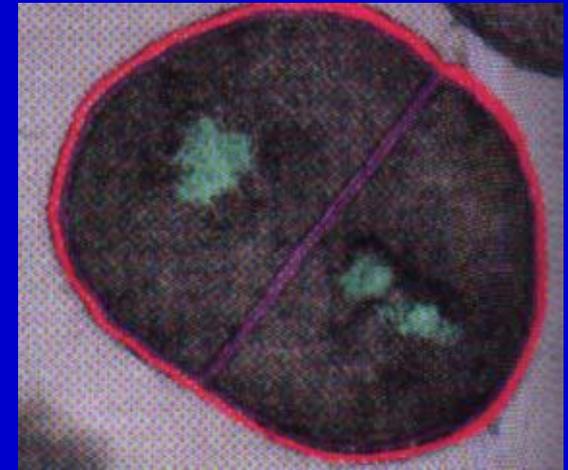
Prokaryotic cell: $G1 \longrightarrow R \longrightarrow D$

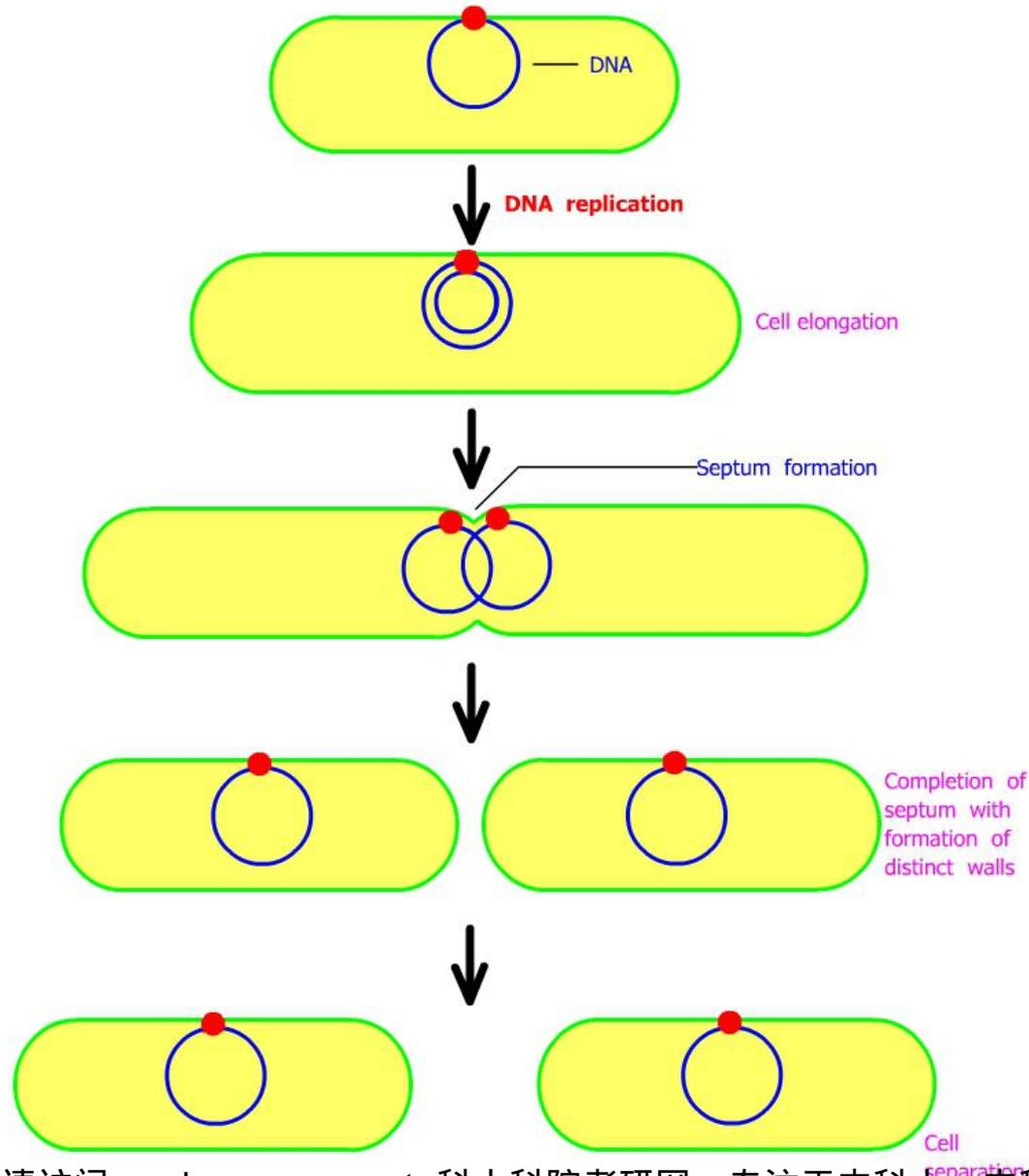
Binary fision

Most bacterial cells reproduce asexually by *binary fision*, a process in which a cell divides to produce two nearly equal-sized progeny cells.

Three processes:

- Increase in cell size (cell elongation)
- DNA replication
- Cell division





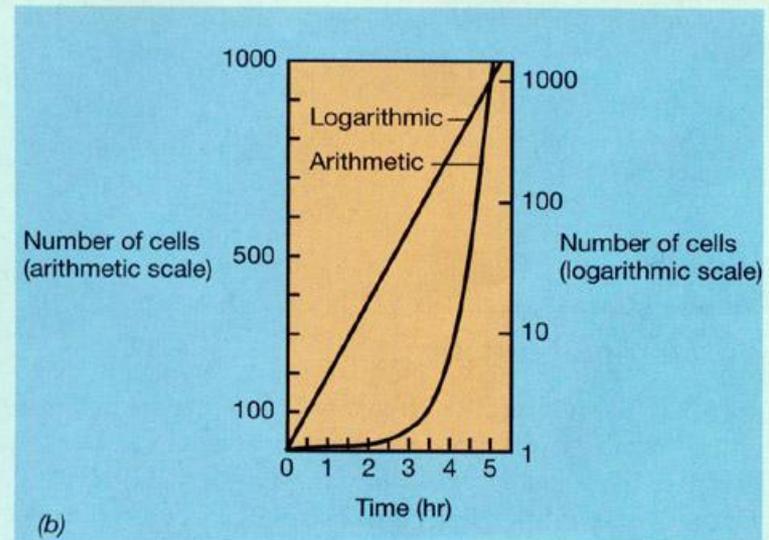
7.2 Population Growth

- Growth is defined as an increase in the number of microbial cells in a population.
- Growth rate is the change in cell number or cell mass per unit time.
- The interval for the formation of two cells from one is called a generation.
- The time required for this to occur is called the generation time.

Exponential Growth

A growth experiment beginning with a single cell having a doubling time of 30 min is presented. This pattern of population increase, where the number of cells doubles during each unit time period, is referred to as exponential growth.

Time (hr)	Total number of cells
0	1
0.5	2
1	4
1.5	8
2	16
2.5	32
3	64
3.5	128
4	256
4.5	512
5	1,024
5.5	2,048
6	4,096
.	.
.	.
(a) 10	1,048,576

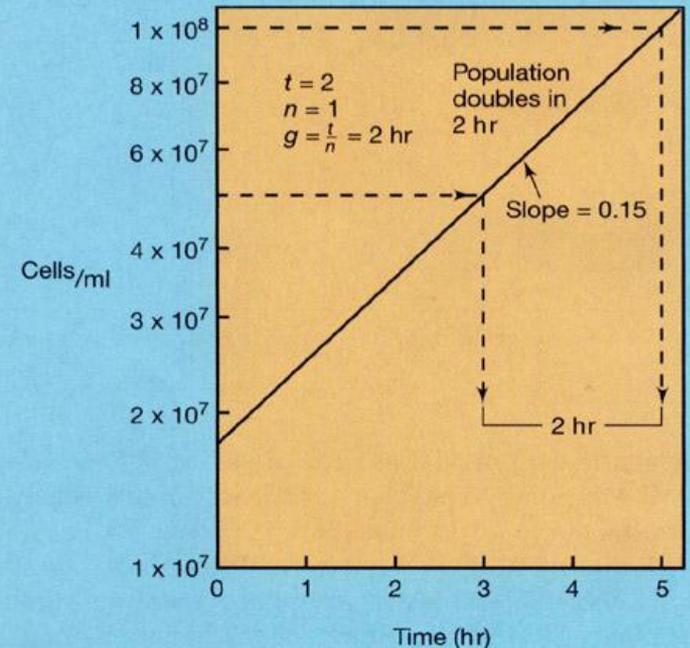
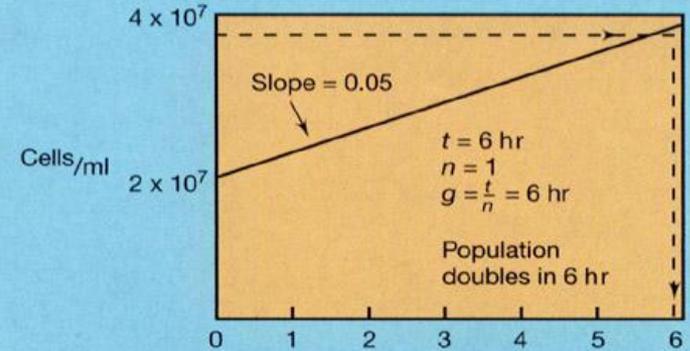


The rate of growth of a microbial culture.
 (a) Data for a population that doubles every 30 min.
 (b) Data plotted on an arithmetic (left ordinate) and a logarithmic (right ordinate) scale.

Calculating Generation Times

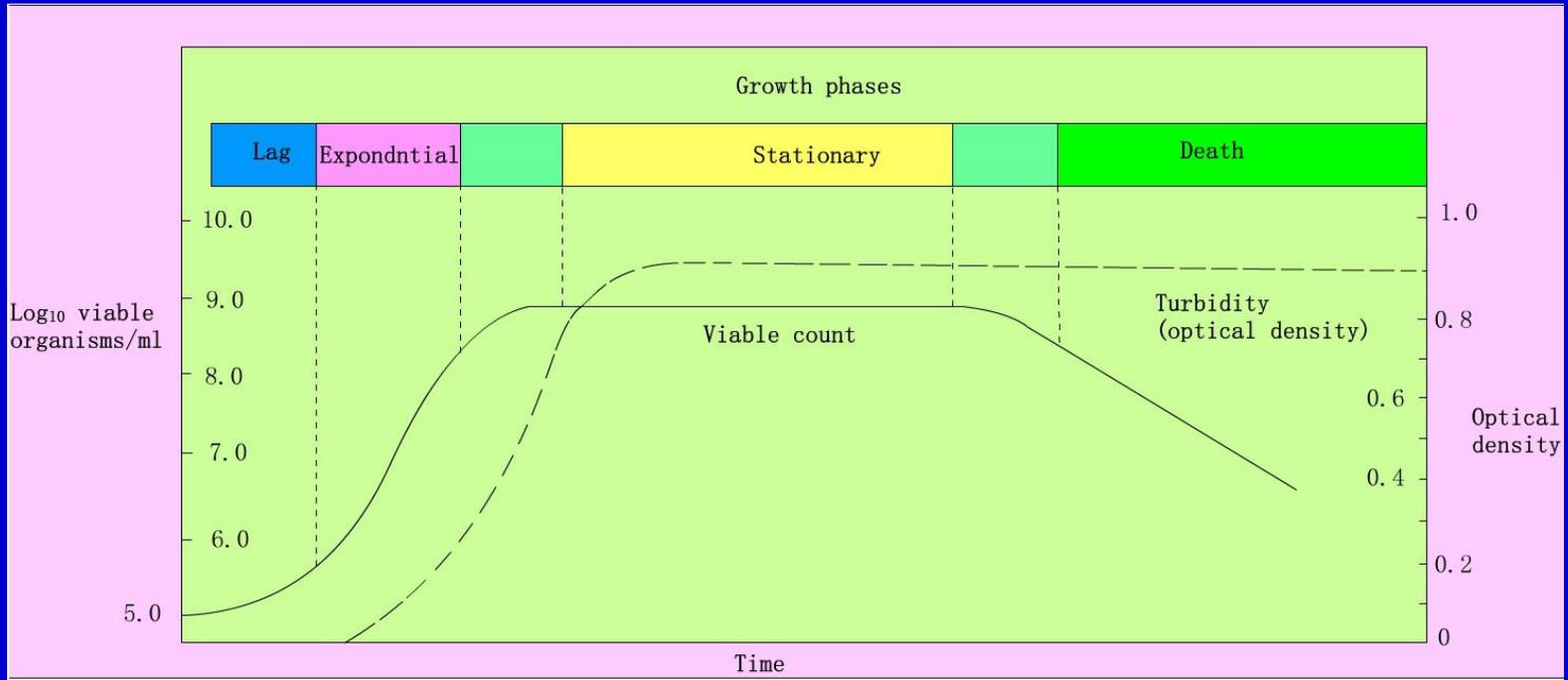
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- $N = N_0 2^n$ N = final cell number. N_0 = initial cell number, and n = number of generations.
- $n = (\log N_0 - \log N) / \log 2 = (\log N_0 - \log N) / 0.301 = 3.3 (\log N - \log N_0)$.
- The generation time g of the cell population is calculated as t / n , where t is simply the hours or minutes of exponential growth.



Method of estimating the generation times (g) of exponentially growing populations with generation times of 6 and 2 hr, respectively, from data plotted on semilogarithmic graphs. The slope of each line is $0.301/g$. All numbers are in scientific notation, that is, 10,000,000 is 1×10^7 , 60,000,000 is 6×10^7 , and so on.

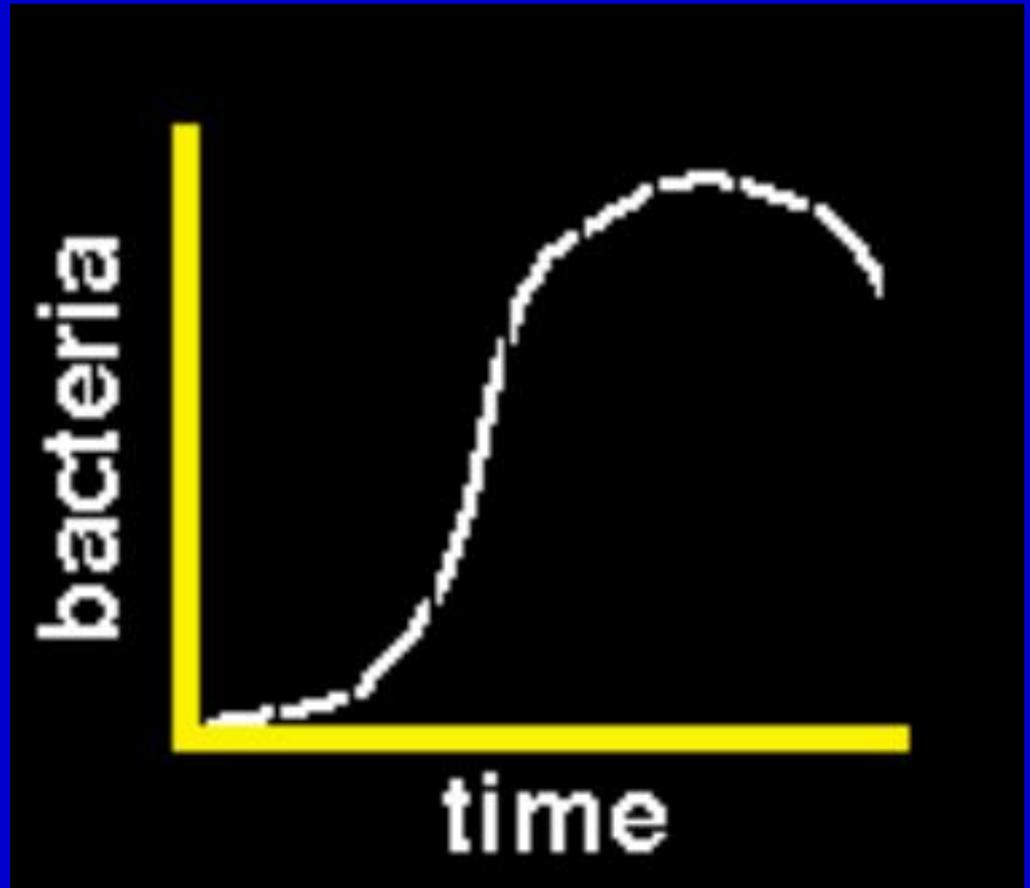
Growth Cycle of Populations



A typical growth curve for a population of cells can be divided into several distinct phases called the lag phase, exponential phase, stationary phase, and death phase.

Growth curve of bacteria

1. Lag Phase
2. Exponential Phase
3. Stationary Phase
4. Death Phase



Lag Phase

When a microbial population is inoculated into a fresh medium, growth usually does not begin immediately but only after a period of time called the lag phase, which may be brief or extended depending on the history of the culture and growth conditions.

This happens because for growth to occur in a particular culture medium the cells must have a complete complement of enzymes for synthesis of the essential metabolites not present in that medium.

Exponential Phase

It is a consequence of the fact that each cell divides to form two cells, each of which also divides to form two more cells, and so on.

Most unicellular microorganisms grow exponentially, but rates of exponential growth vary greatly. In general, prokaryotes grow faster than eukaryotic microorganisms

Stationary Phase

If a single bacterium continued to grow exponentially for 48 hr, produce a population that weighed about 4000 times the weight of Earth! This is particularly impressive because a single bacterial cell weighs only about one-trillionth (10^{-12}) of a gram.

An essential nutrient of the culture medium is used up or some waste product of the organism builds up in the medium to an inhibitory level and exponential growth ceases, or both.

Death Phase

If incubation continues after a population reaches the stationary phase, the cells may remain alive and continue to metabolize, but they may also die. If the latter occurs, the population is said to be in the death phase.

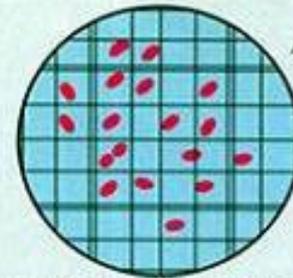
7.3 Measurement of Growth

Population growth is measured by following changes in the number of cells or weight of cell mass.

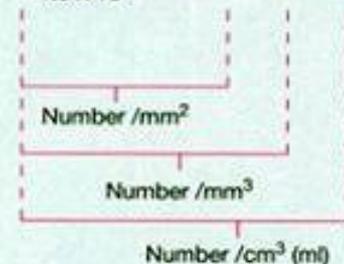
Total Cell Count

The number of cells in a population can be measured by counting a sample under the microscope, either on samples dried on slides or on samples in liquid. With liquid samples, special counting chambers must be used.

Direct microscopic counting procedure using the Petroff-Hausser counting chamber.

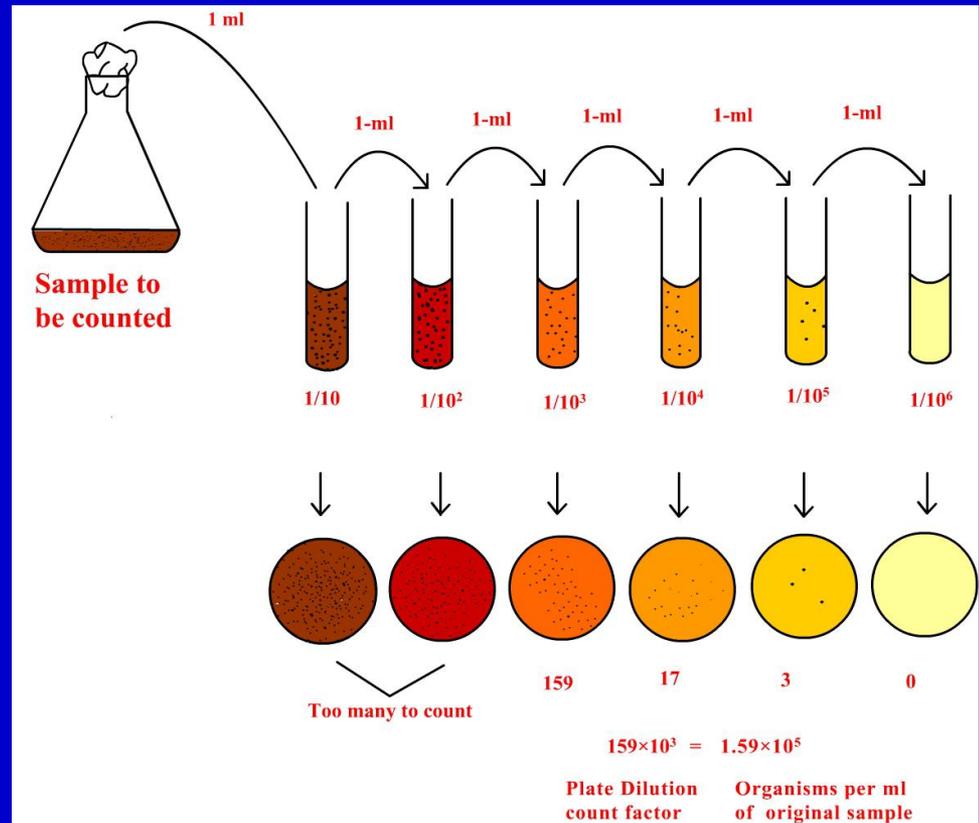


To calculate number per milliliter of sample:
 $12 \text{ cells} \times 25 \text{ squares} \times 50 \times 10^{-3}$
 $= 1.5 \times 10^7$



Viability Counting method

The usual practice, which is the most valid statistically, is to count colonies only on plates that have between 30 and 300 colonies. To make a 10-fold (10^{-1}) dilution, one can mix 0.5 ml of sample with 4.5 ml of diluent, or 1.0 ml sample with 9.0 ml diluent.

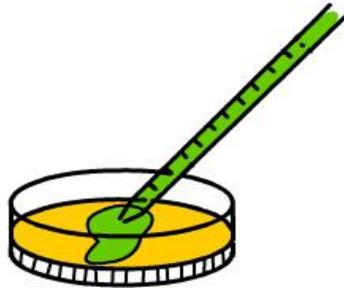


The usual way to perform a viable count is to determine the number of cells in the sample capable of forming colonies on a suitable agar medium.

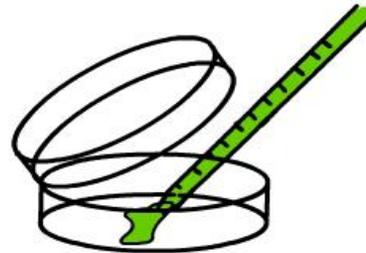
There are two ways of performing a plate count: the spread plate method and the pour plate method. In either case the sample must usually be diluted before plating.

Spread plate

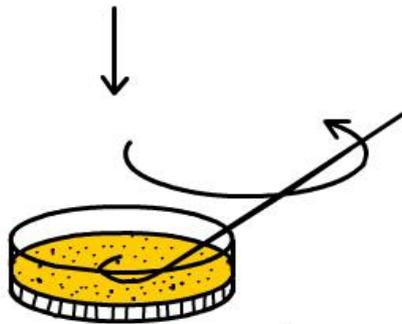
Pour plate



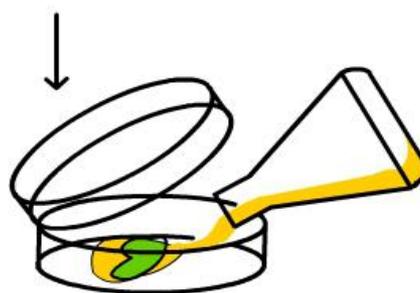
Sample pipetted onto surface of agar plate (0.1 ml or less)



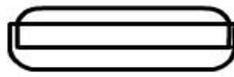
Sample pipetted into sterile plate



Sample is spread evenly oversurface of agar using sterile glass spreader



Sterile medium added and mixed well with inoculum



All plates incubated upside down

Surface colonies



Typical spread -plate results

Surface colonies



Subsurface colonies

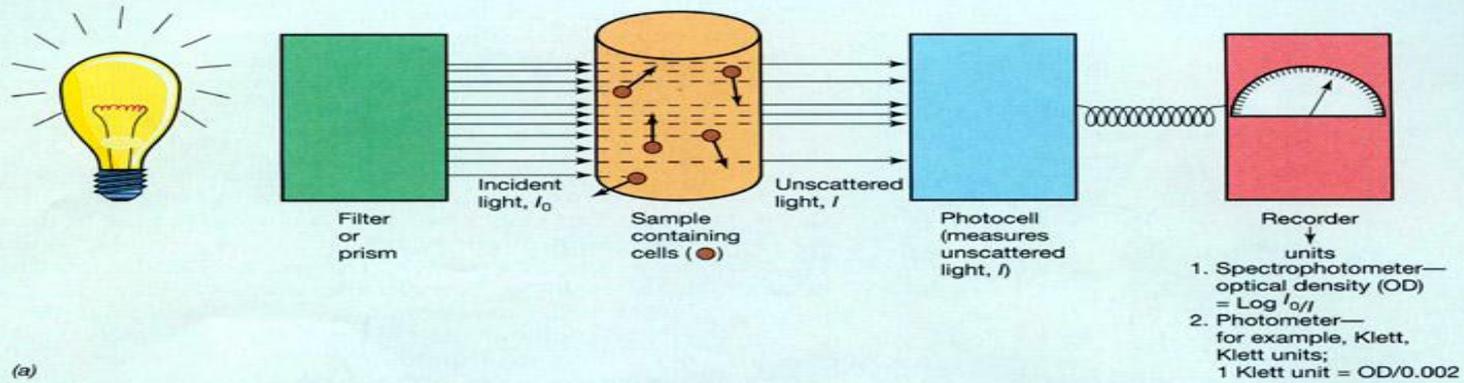
Typical pour-plate results

Sources of Error in Plate Counting

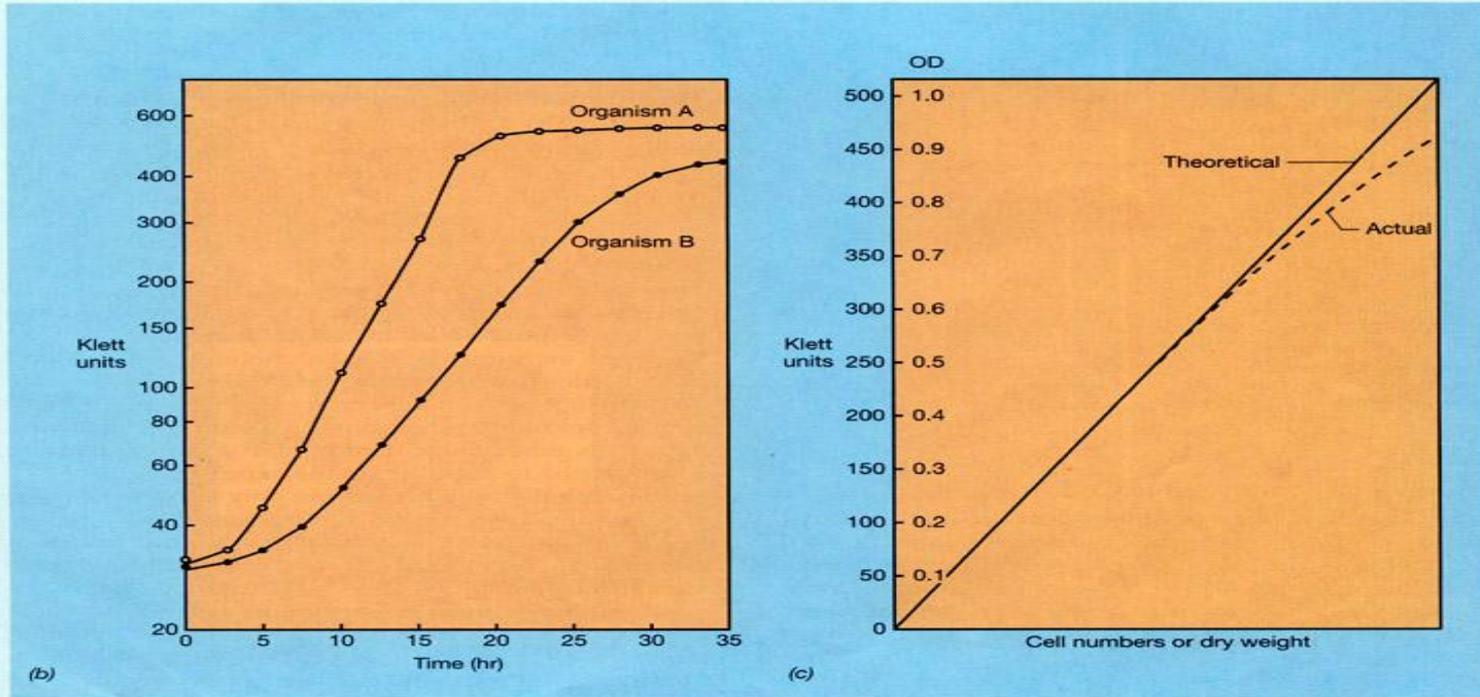
- The number of colonies obtained in a viable count depends not only on the inoculum size but also on the suitability of the culture medium and the incubation conditions used; It also depends on the length of incubation.
- The length of incubation.
- Some tiny colonies may be missed during the counting.
- The incubation conditions (medium, temperature, time).
- Key dilutions must be prepared.

Turbidimetric Measurements of Cell Number

A cell suspension looks cloudy (turbid) to the eye because cells scatter light passing through the suspension. The more cells present, the more light scattered and hence the more turbid the suspension.



(a)



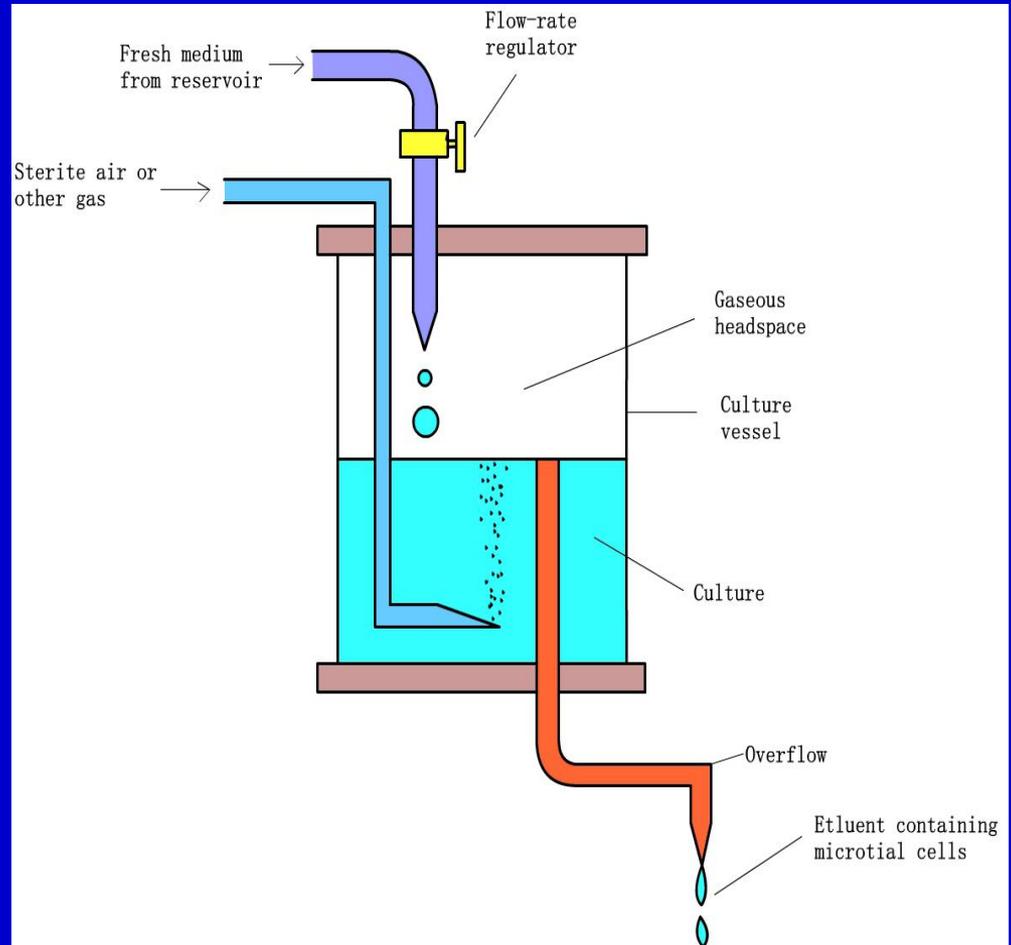
Turbidity measurements of microbial growth. (a) Measurements of turbidity are made in a spectrophotometer or photometer. The photocell measures incident light unscattered by cells in suspension and gives readings in optical density or photometer units. (b) Typical growth curve data obtained in Klett units for two organisms growing at different growth rates. For practice, calculate the generation time (g) of the two cultures using the formula $n = 3.3(\log N - \log N_0)$ where N and N_0 are two different Klett values taken between a time interval t . Which organism is growing faster, A or B? (c) Relationship between cell number or dry weight and turbidity readings. Note that the one-to-one correspondence between these relationships breaks down at high cell numbers.

7.4 Continuous Culture: The Chemostat

A continuous culture is essentially a flow system of constant volume to which medium is added continuously and from which continuous removal of any overflow can occur. Once such a system is in equilibrium, cell number and nutrient status remain constant, and the system is said to be in steady state.

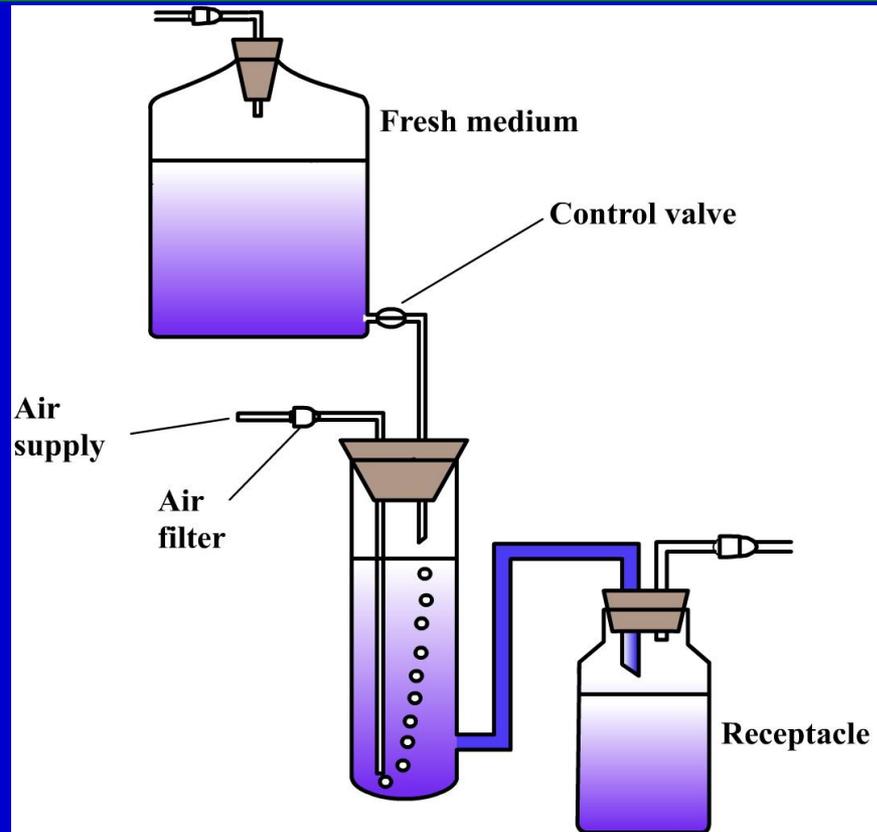
The Chemostat

The most common type of continuous culture device used is a chemostat, which permits control of both the population density and the growth rate of the culture. Both parameters can be set by the experimenter.

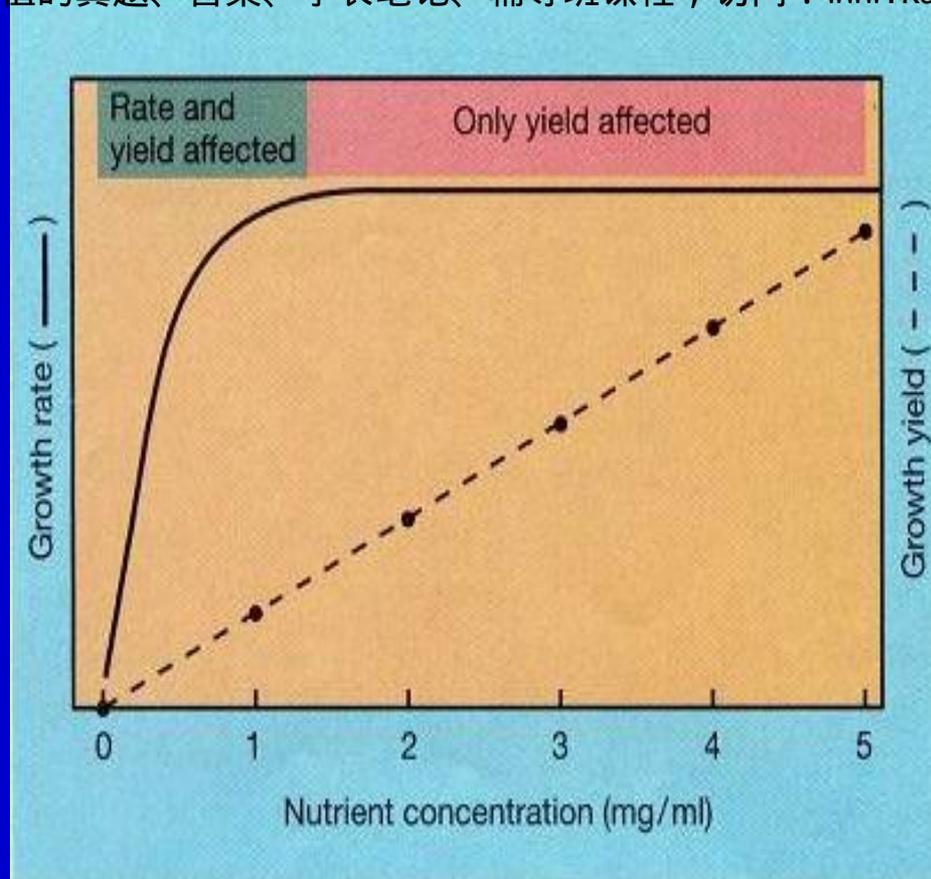


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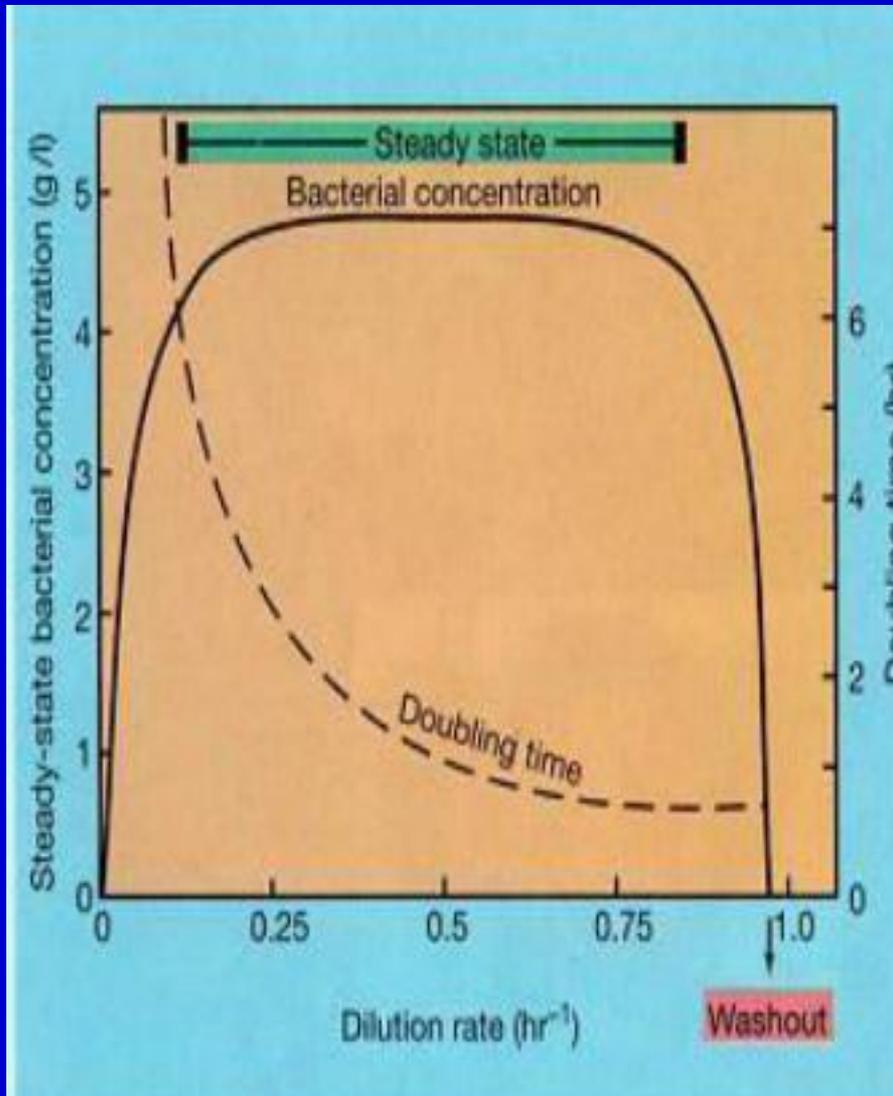
Continuous culture of microorganisms



Chemostat used for continuous cultures. Rate of growth can be controlled either by controlling the rate at which new medium enters the growth chamber or by limiting a required growth factor in the medium.



Relationship among nutrient concentration, growth rate(solid line),and growth yield(dashed line)in a batch culture(closed system). At low nutrient concentrations both growth rate and growth yield are affected.



Steady-state relationships in the chemostat. The dilution rate is determined from the flow rate and the volume of the culture vessel. Thus, with a vessel of 1000ml and a flow rate through the vessel of 500ml/hr, the dilution rate would be 0.5hr⁻¹.

Note that at high dilution rates, growth cannot balance dilution, and the population washes out.

Note also that although the population density remains constant during steady state, the growth rate (doubling time) can vary over a wide range. Thus, the experimenter can obtain populations with widely varying growth rates without affecting population density.

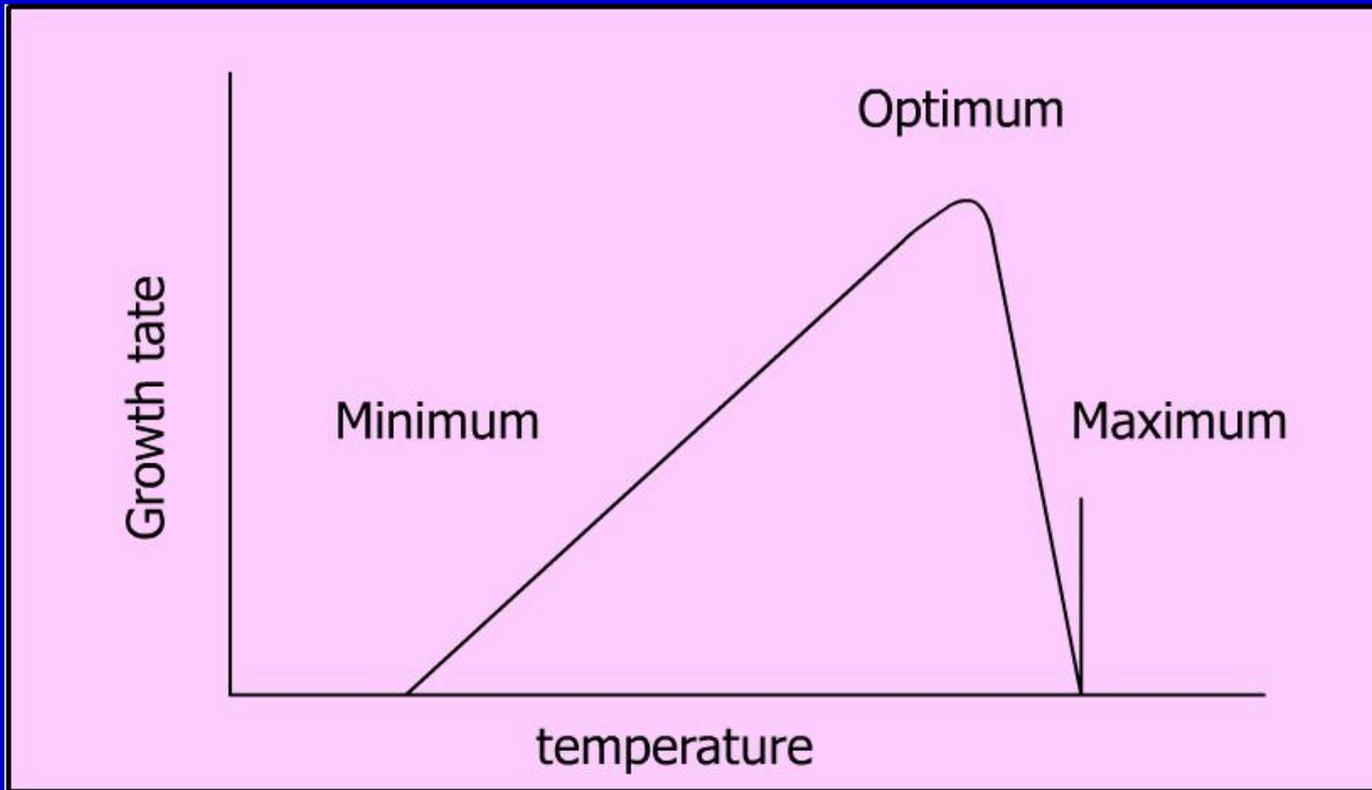
Experimental Uses of the Chemostat

- Even over rather wide ranges, any desired growth rate can be obtained in the chemostat by simply varying the dilution rate.
- A practical advantage to the chemostat is that a population may be maintained in the exponential growth phase for long periods, for days and even weeks. The experimenter using the chemostat can have such cells available at any time.

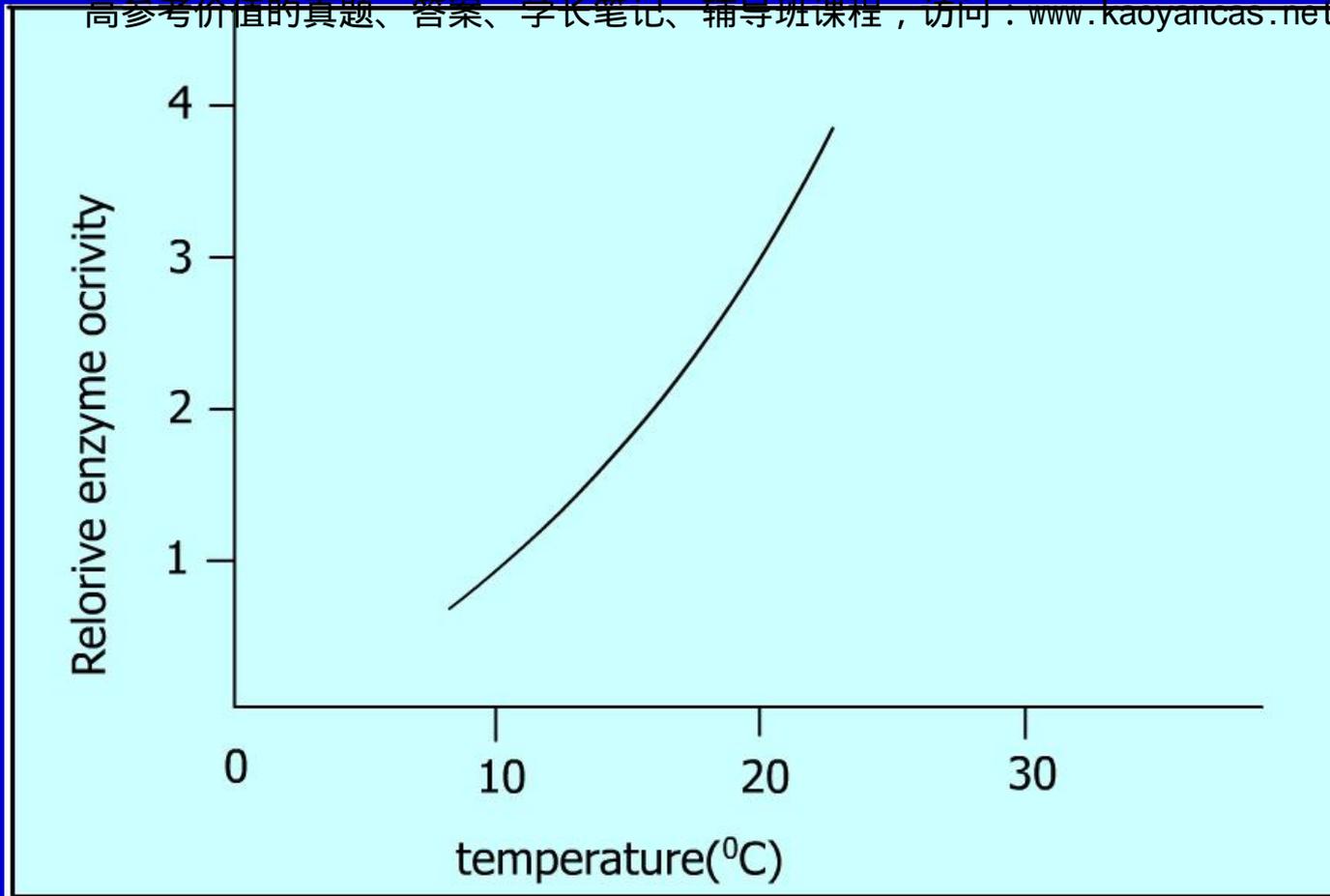
7.5 Effect of Environment on Growth

- The activities of microorganisms are greatly affected by the chemical and physical conditions of their environments. Understanding environmental influences helps us to explain the distribution of microorganisms in nature and makes it possible for us to devise methods for controlling microbial activities and destroying undesirable organisms.
- **Four main factors :**
Temperature, pH, water availability, and oxygen.

Effect of temperature on bacterial growth rate

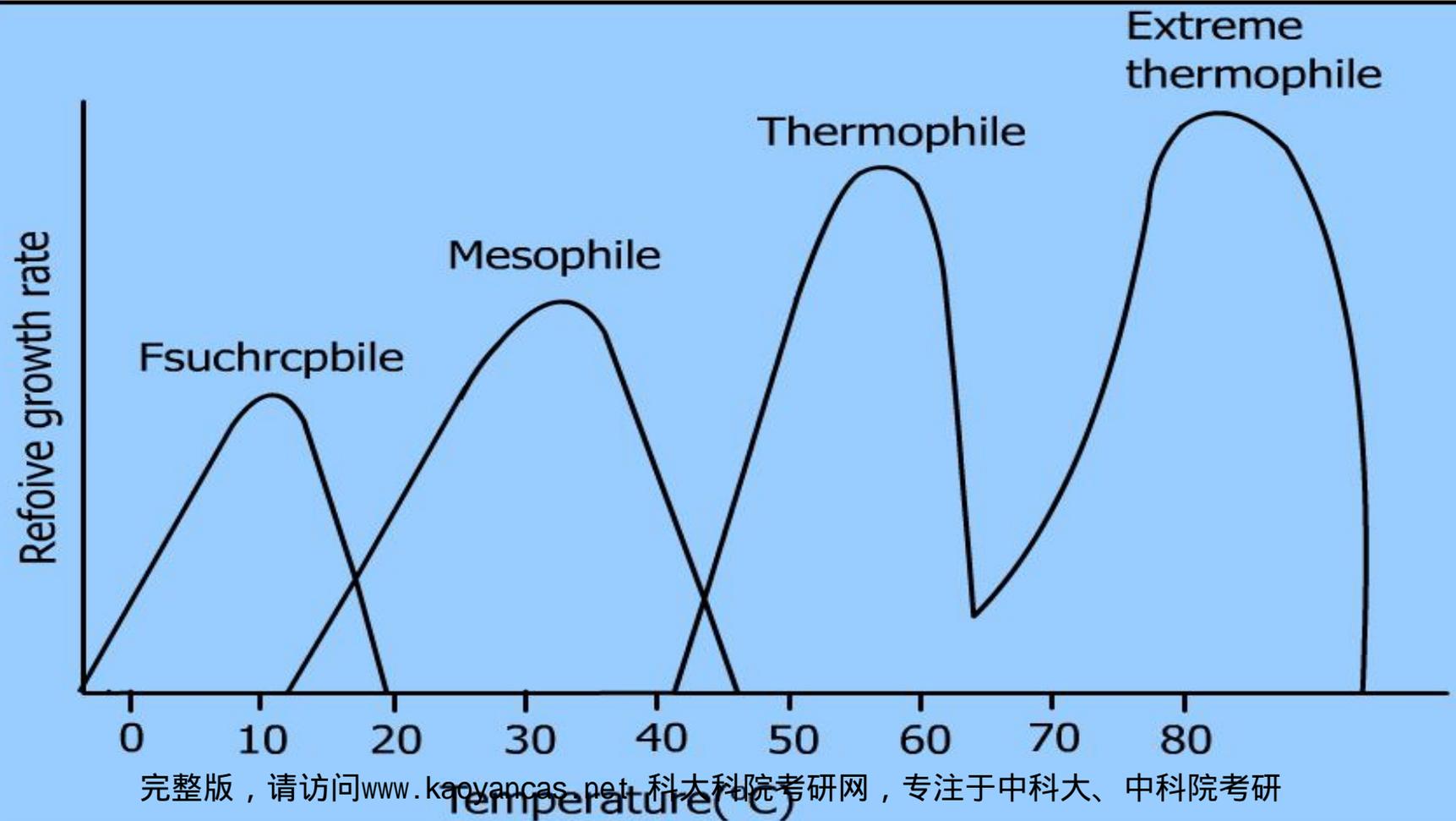


Bacteria grow over a range of temperatures; they do not reproduce below the minimum growth temperature nor above the maximum growth temperature. Within the temperature growth range there is an optimum growth temperature at which bacterial reproduction is fastest.

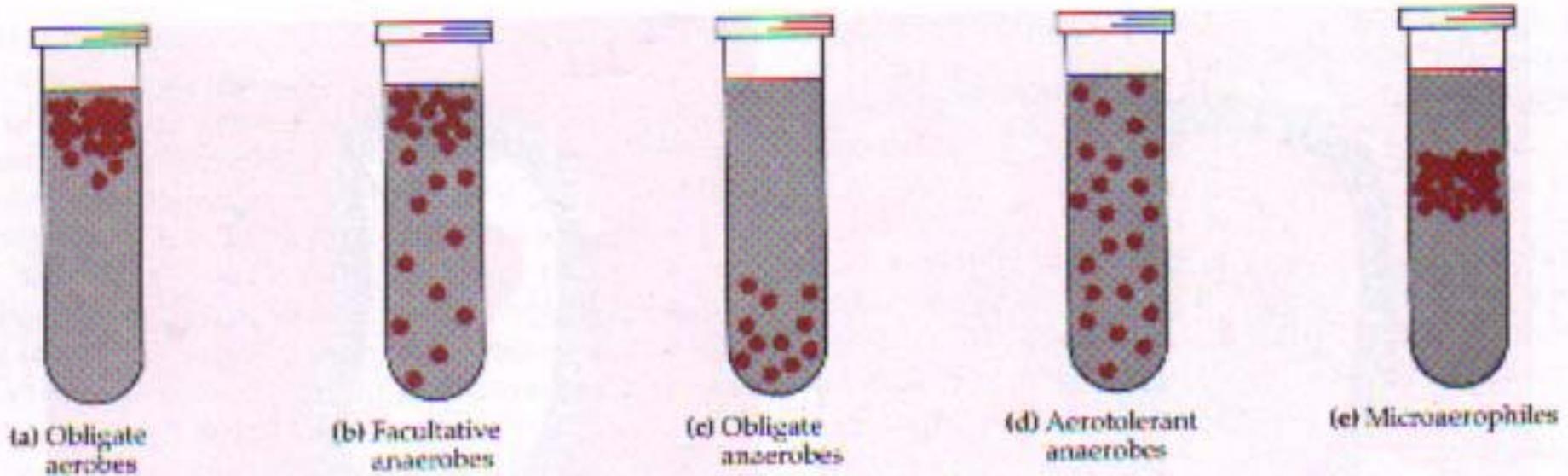


Enzymes exhibit a Q_{10} so that within a suitable temperature range the rate of enzyme activity doubles for every 10° C rise in temperature.

Microorganisms are classified as psychrophiles, mesophiles, thermophiles, and extremethemophiles based on their optimal growth temperature.



Effect of oxygen concentration on the growth of various bacteria in tubes of solid medium.



(a) Obligate aerobes grow with oxygen, usually in the thin surface layer where oxygen is high, but do not tolerate high oxygen concentrations.
(b) Facultative anaerobes grow with or without oxygen, but prefer to grow in the oxygen-rich surface.
(c) Obligate anaerobes do not tolerate oxygen at all and grow only in the absence of oxygen.
(d) Aerotolerant anaerobes do not require oxygen and grow uniformly throughout the tube.
(e) Microaerophiles require a low concentration of oxygen and grow in a thin layer in the middle of the tube.

Bacteria → Neutral condition

Fungi → Acidic condition

Actinomycetes → Alkaline condition

Water activity

The water activity of a solution is $1/100$ the relative humidity of the solution (when expressed as a percent), or it is equivalent to the ratio of the solution's vapor pressure to that of pure water.

$$a_w = P_{\text{solution}} / P_{\text{water}}$$

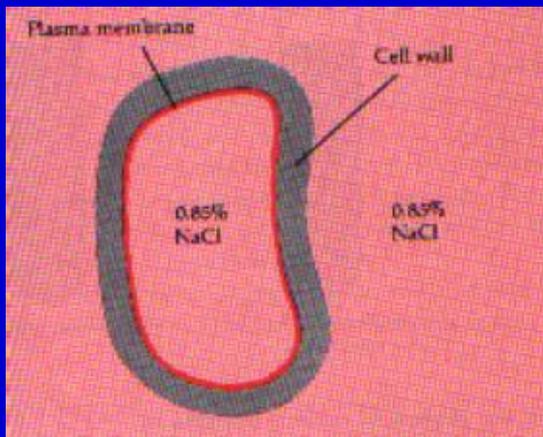
Approximate lower a_w limits for microbial growth:

- 0.90 – 1.00 for most bacteria, most algae and some fungi as *Basidiomycetes*, *Mucor*, *Rhizopus*.
- 0.75 for *Halobacterium*, *Aspergillus*...
- 0.60 for some *saccharomyces* species

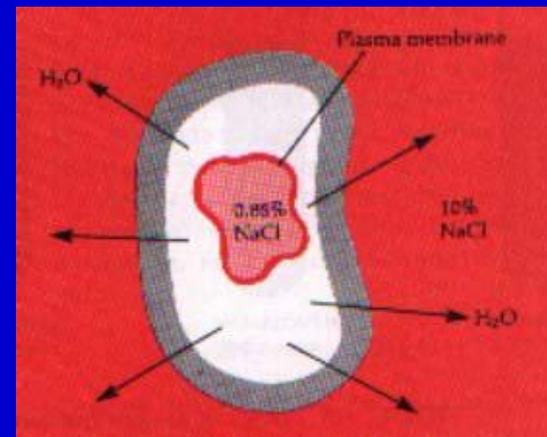
Water activity

If the concentration of solutes, such as sodium chloride, is higher in the surrounding medium (hypertonic), then water tends to leave the cell. The cell membrane shrinks away from the cell wall (an action called plasmolysis), and cell growth is inhibited.

Normal cell



Plasmolyzed cell



7.6 Growth Control

Definitions:

Sterilization – the process of destroying all forms of microbial life on an object or in a material.

Disinfection – the process of destroying vegetative pathogens but not necessary endospores.

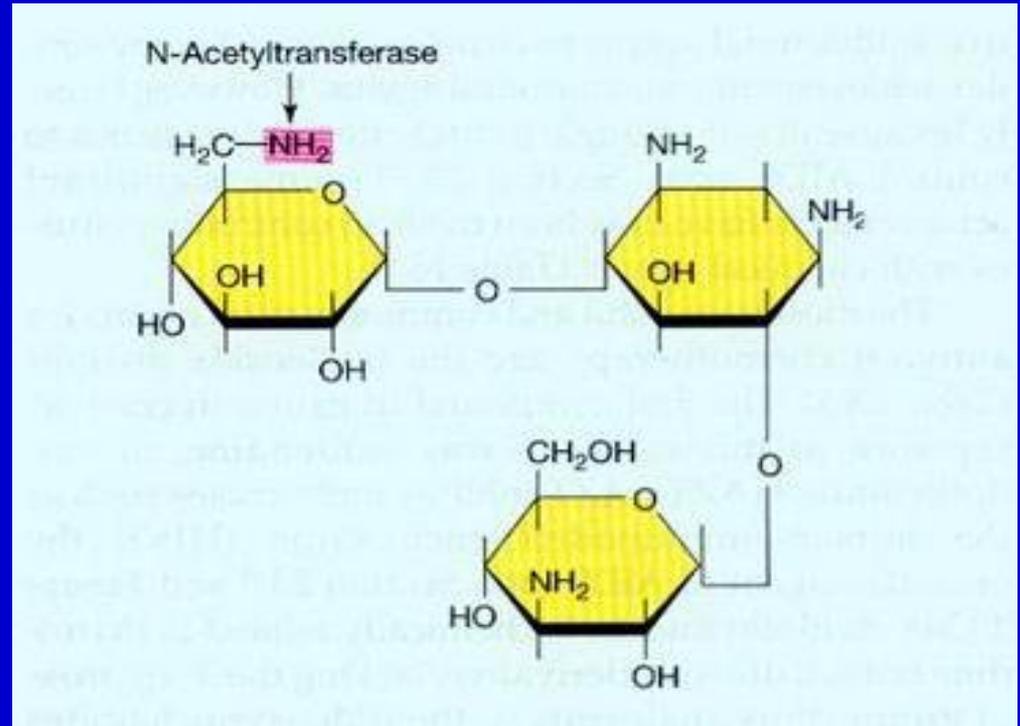
Antisepsis – chemical disinfection of skin, mucous membranes or other living tissues

Prokaryote Control

- Many antibiotics active against prokaryotes are also produced by prokaryotes.
- Include the aminoglycosides, the macrolides, and the tetracyclines. Many of these antibiotics have major clinical applications.
- The tetracyclines and the β -lactam antibiotics are the two most important groups of antibiotics in the medical field.

Aminoglycoside antibiotics

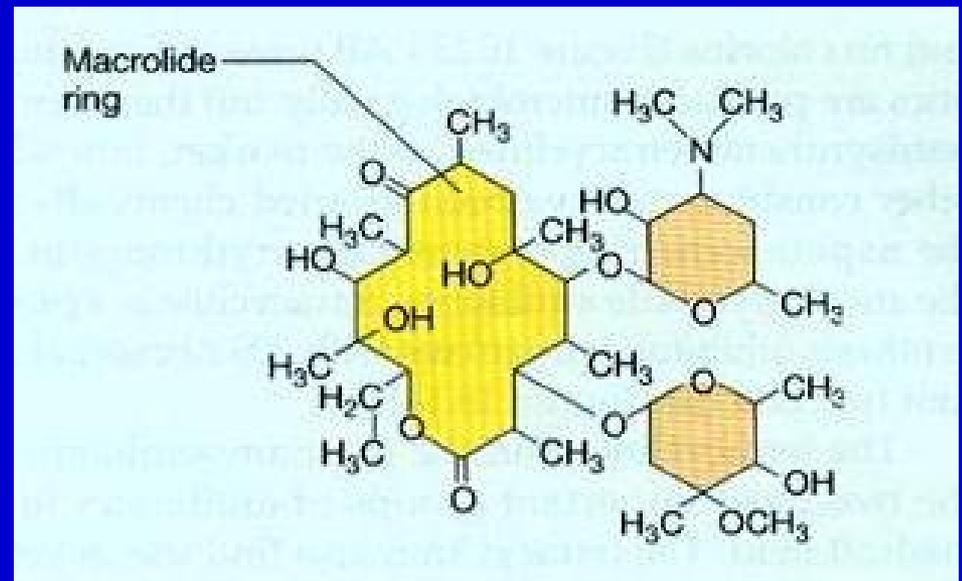
- Contain amino sugars bonded by glycosidic linkage to other amino sugars .
- Inhibiting protein synthesis at the 30S subunit of the ribosome.
- Including streptomycin and its relatives, kanamycin, gentamicin, and neomycin , are used clinically against gram-negative Bacteria.



Structure of **kanamycin**, an aminoglycoside antibiotic. The site of modification by an *N*-acetyltransferase, encoded by a resistance plasmid, is indicated.

Macrolide antibiotics

- Contain large lactone rings connected to sugar moieties.
- Include erythromycin, oleandomycin, spiramycin, and tylosin.
- Acts as a protein synthesis inhibitor at the level of the 50S subunit of the ribosome.



Structure of **erythromycin**, a typical macrolide antibiotic.

Tetracyclines

- The tetracyclines inhibiting almost all gram-positive and gram-negative Bacteria.
- Tetracycline is a protein synthesis inhibitor.
- It interferes with 30S ribosomal subunit function.



Tetracycline form	R ₁	R ₂	R ₃	R ₄
Tetracycline	H	OH	CH ₃	H
7-Chlortetracycline (aureomycin)	H	OH	CH ₃	Cl
5-Oxytetracycline (terramycin)	OH	OH	CH ₃	H

Structure of **tetracycline** and important derivatives.

7.7 Viral Control

- Viruses actually use the host cell machinery to perform their metabolic functions. Therefore, most attempts at chemical control of viruses result in toxicity for the host.
- Several agents are more toxic for the virus than the host, and there are a few agents produced by the host that specifically target viruses.
- There are several classes of chemotherapeutic agents that have been shown to be clinically effective in controlling viral replication.

Antiviral Chemotherapeutic Agents

- The most successful and commonly used agents for antiviral chemotherapy are the nucleoside analogs. The first compound to gain universal acceptance in this category was azidothymidine(AZT), Azidothymidine is chemically related to thymidine but is a dideoxy derivative, AZT inhibits HIV. Because the normal host cell function of DNA replication is targeted, these drugs almost always exhibit some level of host toxicity.
- Several other chemicals work at the level of viral polymerase.
- A relatively novel class of antiviral drugs are the protease inhibitors.

Antiviral chemotherapeutic compounds

Category/drug	Mechanism of action	Virus affected	
Nucleoside analogs			
Acyclovir	Viral polymerase inhibitors	Herpes viruses, <i>Varicella zoster</i>	
Gancyclovir		Cytomegalovirus	
Trifluridine		Herpesvirus	
Valacyclovir		Herpesvirus	
Vidarabine		Herpesvirus, vaccinia, hepatitis B virus	
Didanosine (dideoxyinosine or ddI)		Reverse transcriptase inhibitors	HIV ^a
Lamivudine (3TC)	HIV, hepatitis B virus		
Stavudine (d4T)	HIV		
Zalcitabine (ddC)	HIV		
Zidovudine (AZT)	HIV (∞ Figure 23.34)		
Ribavirin	Blocks capping of viral RNA		Respiratory syncytial virus, influenza A and B, Lassa fever
Synthetic amines			
Amantadine	Block uncoating of virus	Influenza A	
Rimantadine		Influenza A	
Nucleotide analog			
Cidofovir	Viral polymerase inhibitor	Cytomegalovirus, herpesviruses	
Pyrophosphate Analog			
Phosphonoformic acid	Viral polymerase inhibitor	Herpesviruses, HIV, hepatitis B virus	
Nonnucleoside reverse transcriptase inhibitor			
Nevirapine	Reverse transcriptase inhibitor	HIV	
RNA polymerase inhibitor			
Rifamycin	RNA polymerase inhibitor	Vaccinia, pox viruses	
Protease inhibitors			
Indinavir	Protease inhibitors	HIV	
Ritonavir		HIV	
Saquinavir		HIV	
Nelfinavir		HIV	
Interferons			
Interferon α	Induces proteins that inhibit viral replication	Broad spectrum (host specific)	
Interferon β			
Interferon γ			

Interferon

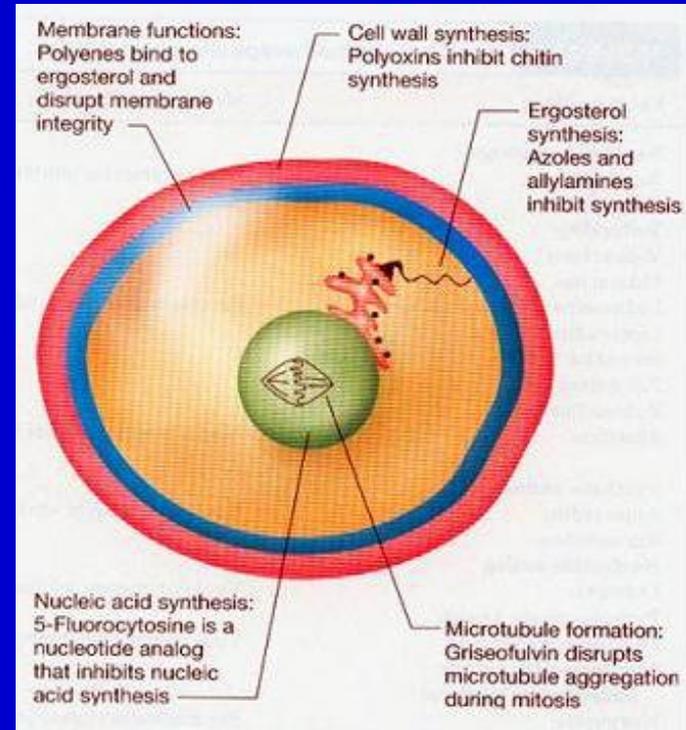
- Interferons are antiviral substances produced by many animal cells in response to infection by certain viruses. They are low-molecular-weight proteins (17,000 MW) that prevent viral multiplication in normal cells by stimulating the production of antiviral proteins.
- There are three molecular types, IFN- α , produced by leukocytes; IFN- β , produced by fibroblasts; and IFN- γ , produced by immune cells known as lymphocytes.

7.8 Fungal Control

- Since fungi are Eukarya, much of their cellular machinery is the same as in higher animals and humans, and so chemotherapeutic agents that affect metabolic pathways in fungi often affect corresponding pathways in host cells. This results in drug toxicity in higher animals. Thus, many antifungal drugs can be used only for topical (surface) applications.
- Some drugs are selectively toxic for fungi. Drugs for fungal treatment are becoming increasingly important as fungal infections in immunosuppressed individuals become more prevalent.

Ergosterol Inhibitors

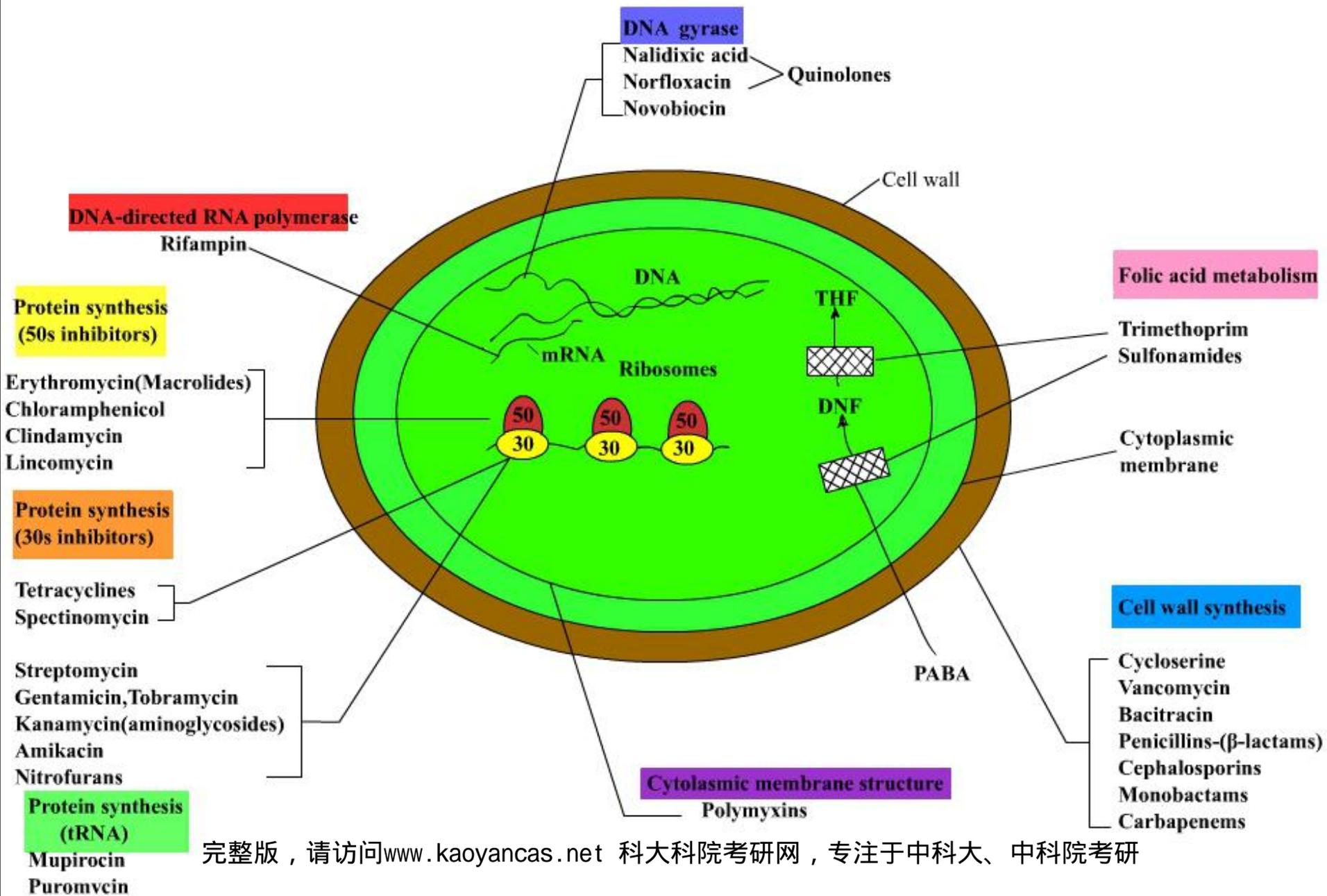
Two major groups of antifungal compounds work by interacting with ergosterol or inhibiting its synthesis. In most fungi, ergosterol replaces the cholesterol component found in higher eukaryotic cell membranes.



Sites of action of some antifungal chemotherapeutic agents. Because fungi are eukaryotic cells, antibacterial antibiotics are generally ineffective.

mode of Action of Major Antibacterial Antibiotics

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Other Antifungal Agents

- A number of other antifungal drugs interfere with fungus-specific structures and functions. Several drugs such as the polyoxins inhibit cell wall synthesis by interfering with chitin biosynthesis.
- Other drugs inhibit folate biosynthesis, interfere with DNA topology during replication, or, like griseofulvin, disrupt microtubule aggregation during mitosis.
- Some very effective antifungal drugs also have other biological applications. For example, vincristine, vinblastin, and taxol are effective antifungal agents and have known anticancer properties.



REVIEW QUESTIONS

1. What is the difference between the growth rate constant (k) of an organism and its generation time (g)?
2. Describe the growth cycle of a population of bacterial cells from the time this population is first inoculated into fresh medium. How can the growth pattern differ when it is measured by total count or by viable count?

3. Describe briefly the process by which a single cell develops into a visible colony on an agar plate. With this explanation as a background, describe the principle behind the viable count method.
4. How can a chemostat regulate growth rate and cell numbers independently?
5. List three chemical classes of compatible solutes produced by various microorganisms. List at least two things they all have in common.

6. Concerning the pH of the environment and of the cell, in what ways are acidophiles and alkaliphiles different? In what ways are they similar?
7. What tests would you perform to decide whether a chemical agent could be used as an antiseptic? As a disinfectant? Some chemicals serve both purposes. Describe the properties of such a chemical and give an example.