Chapter 6

微生物的生长与控制

Microbial Growth and Control

Contents

1 Detection Method

2 Growth Curve

3 Main Factors Impact Microbial Growth

4 Cultivation of Microbes

5 Control the Harmful Microbes

1 Detection Method

- **Measuring the biomass (生物量)**

  (1) Direct method:

  dry mass weighing

  (2) Indirect method:

  turbidimetry;

  Physiological index

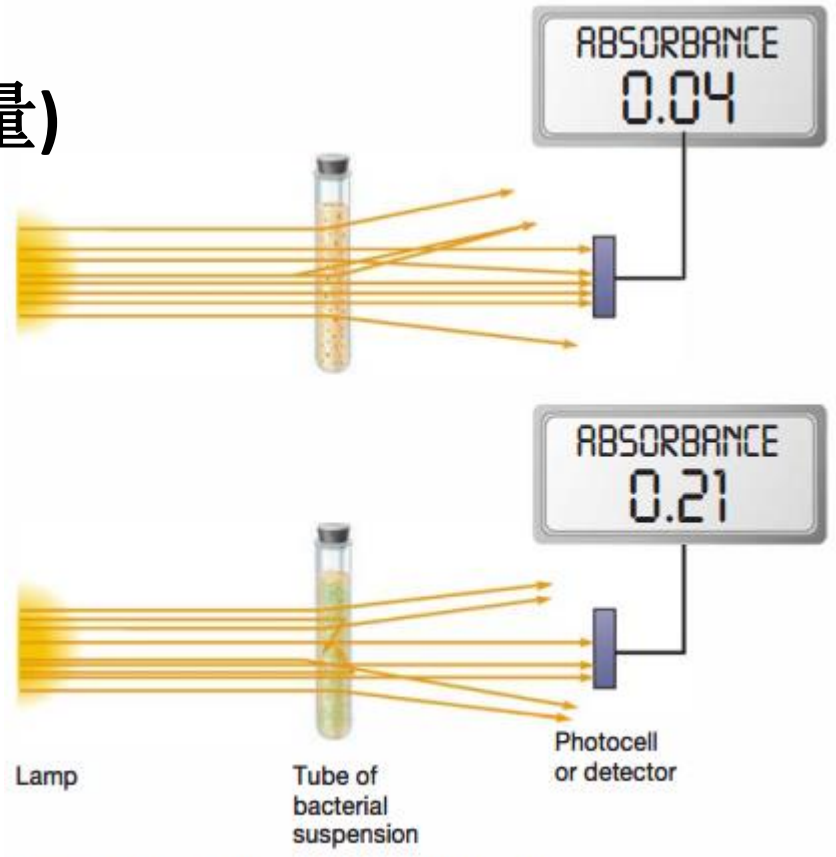
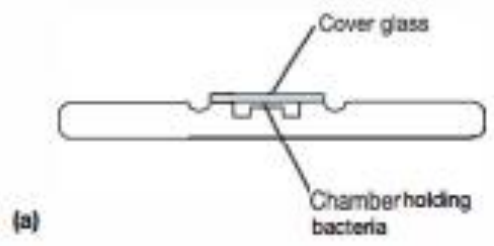
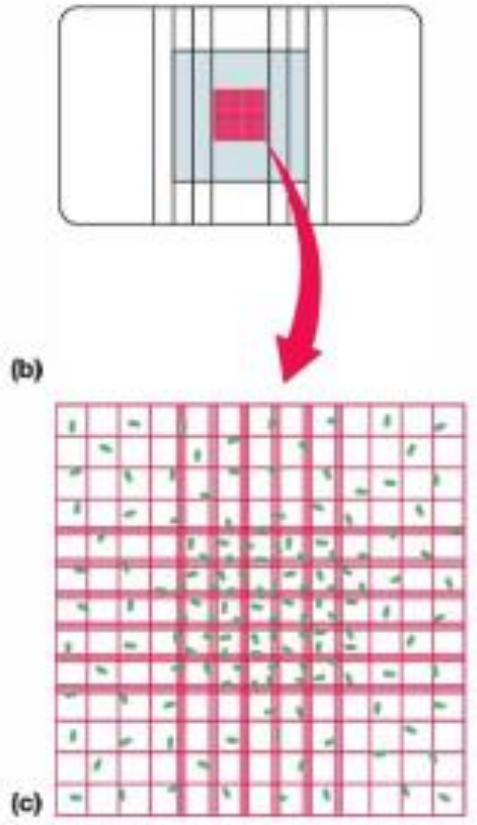
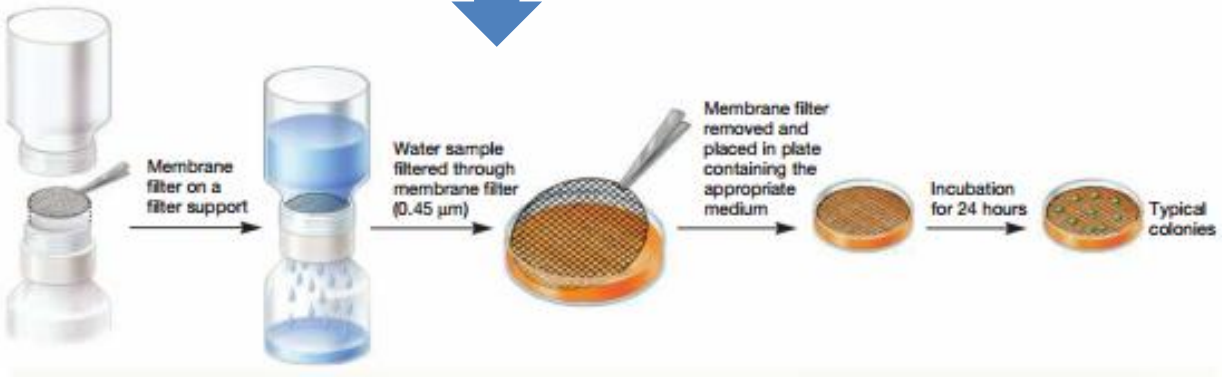


Figure 7.37 Turbidity and Microbial Mass Measurement.

- ● **Countering cell number**
- (1) Direct method:
- Vital staining
- (2) Indirect method:
- Plate colony counting for:
- Anaerobe (discuss late)
- aerobic bacteria







- **Pour plate technique/倾注平板法**

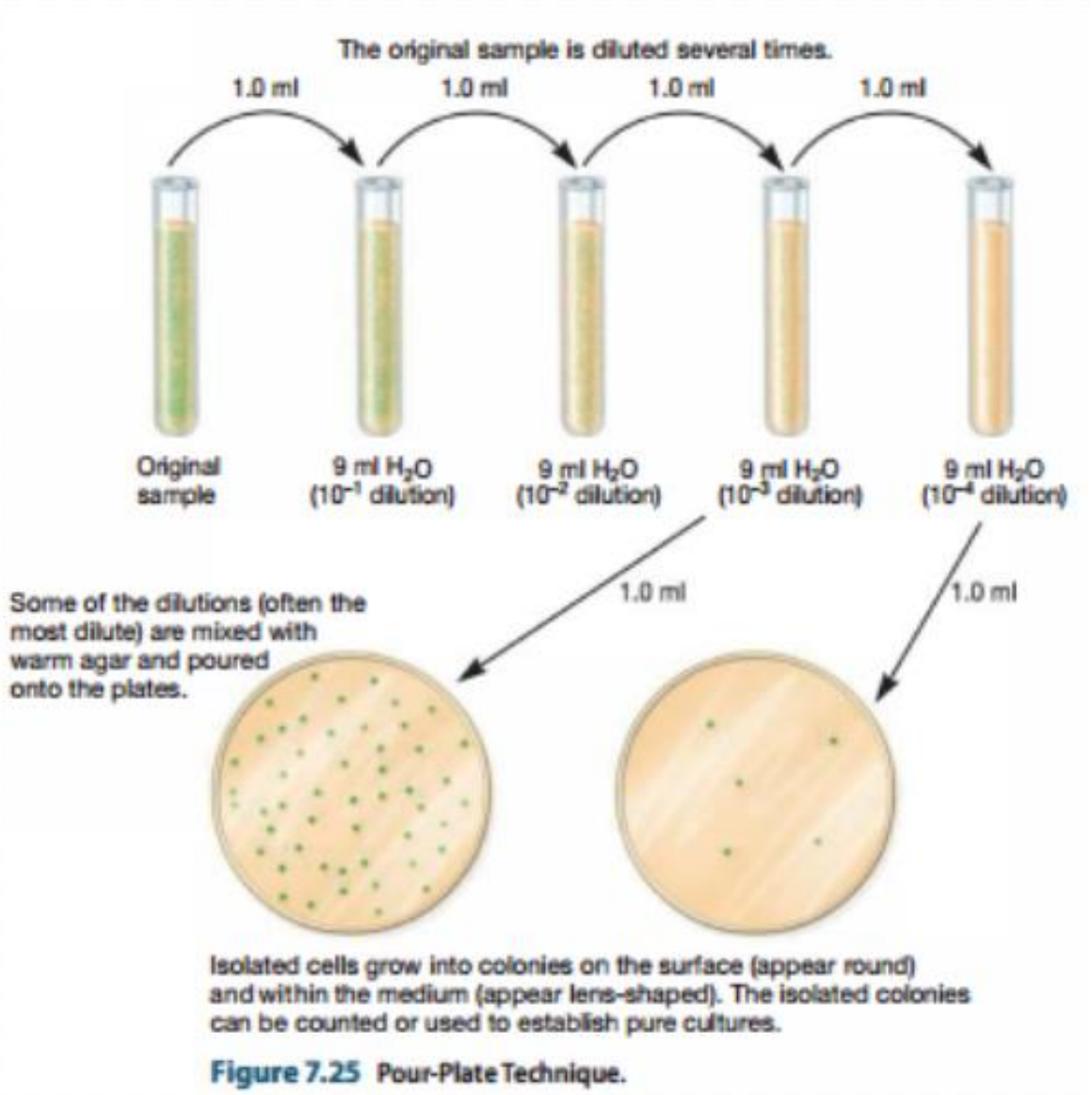
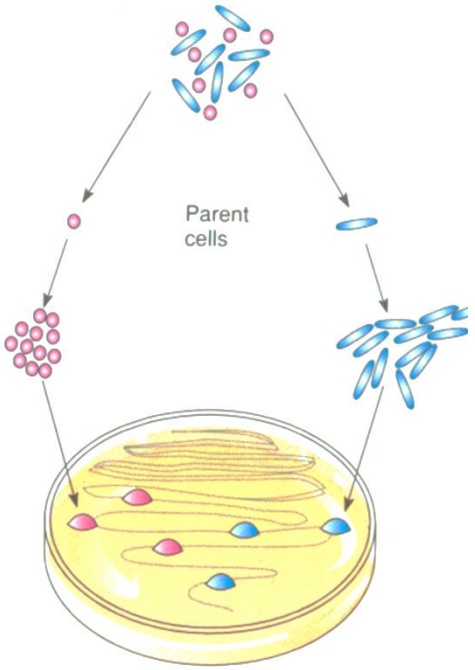


Figure 7.25 Pour-Plate Technique.



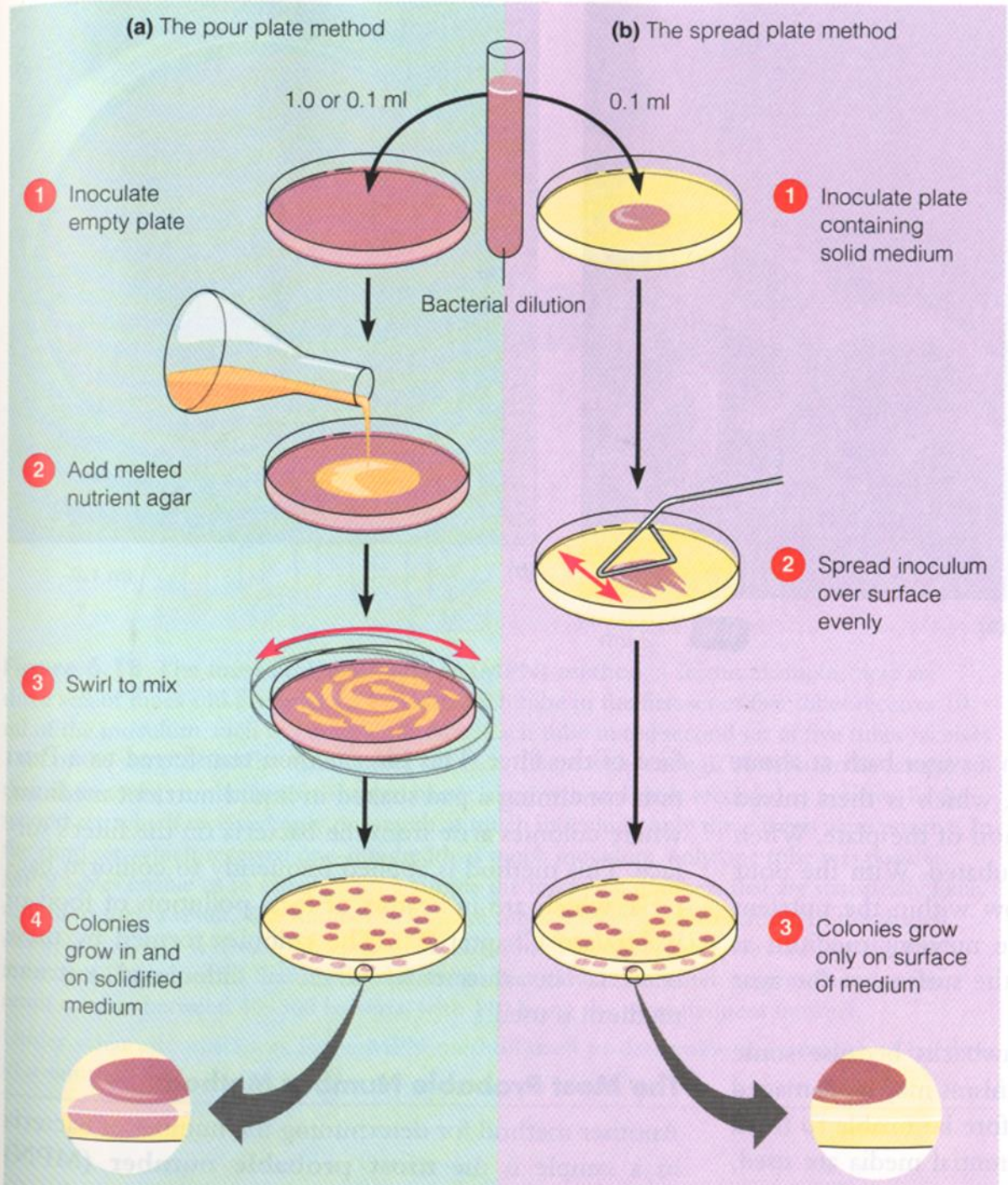


Figure 6.16 Methods of preparing plates for plate counts.

How do pour plates and spread plates differ?

2

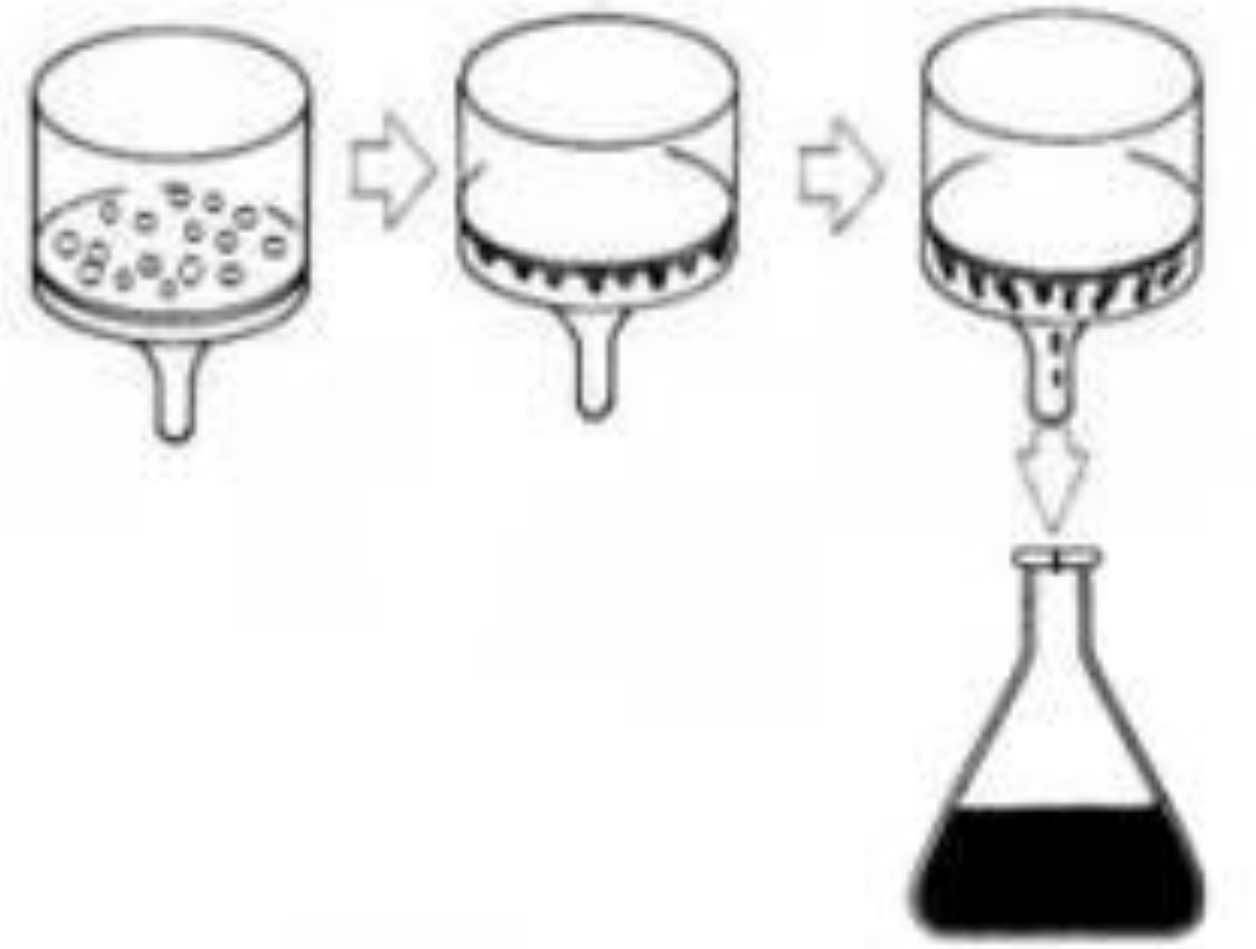
Growth Curve

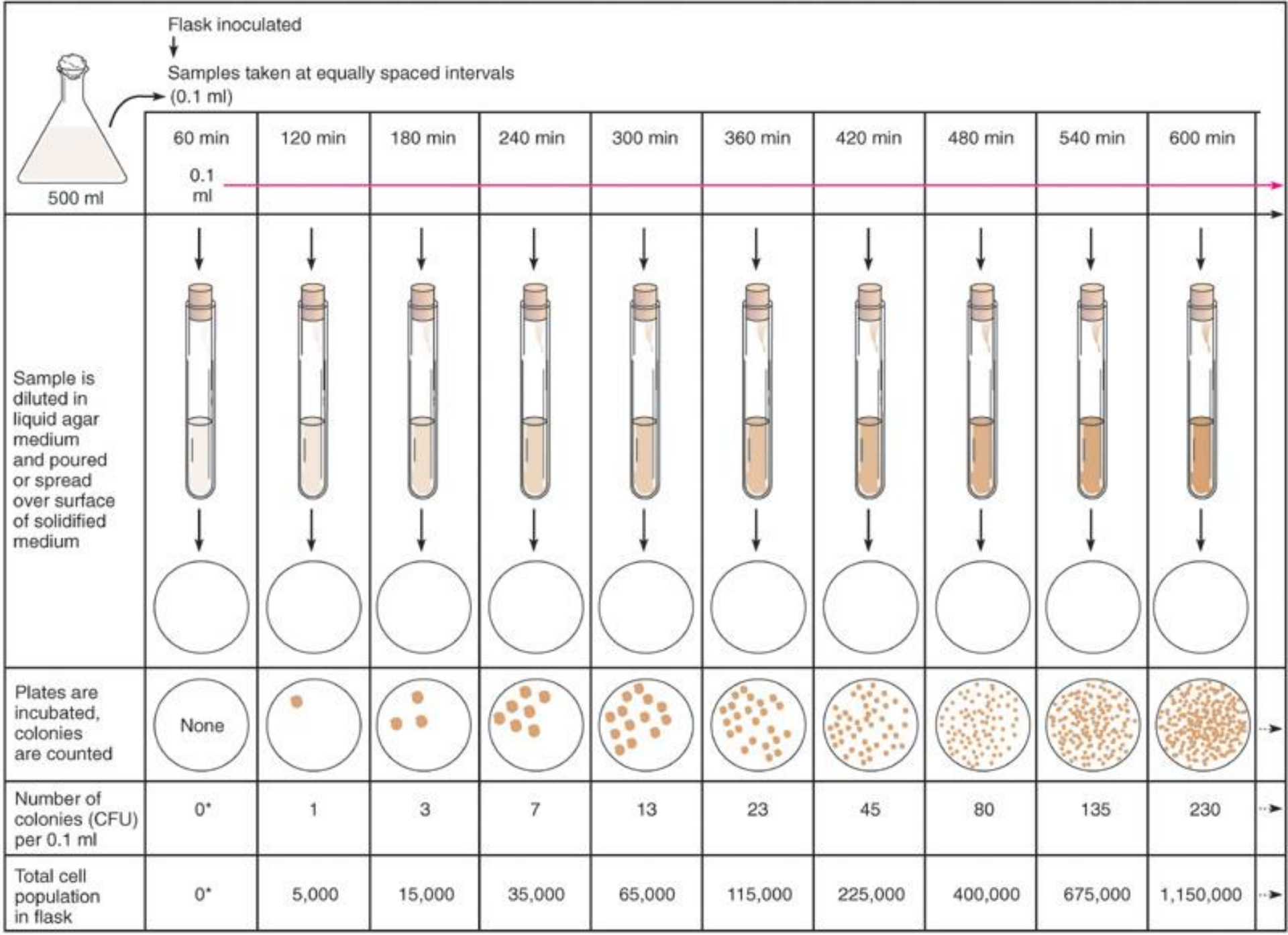
2.1 Synchronous growth/ 同步生长

(1) Environmental condition induction method

(2) Mechanical screening/机械筛选法

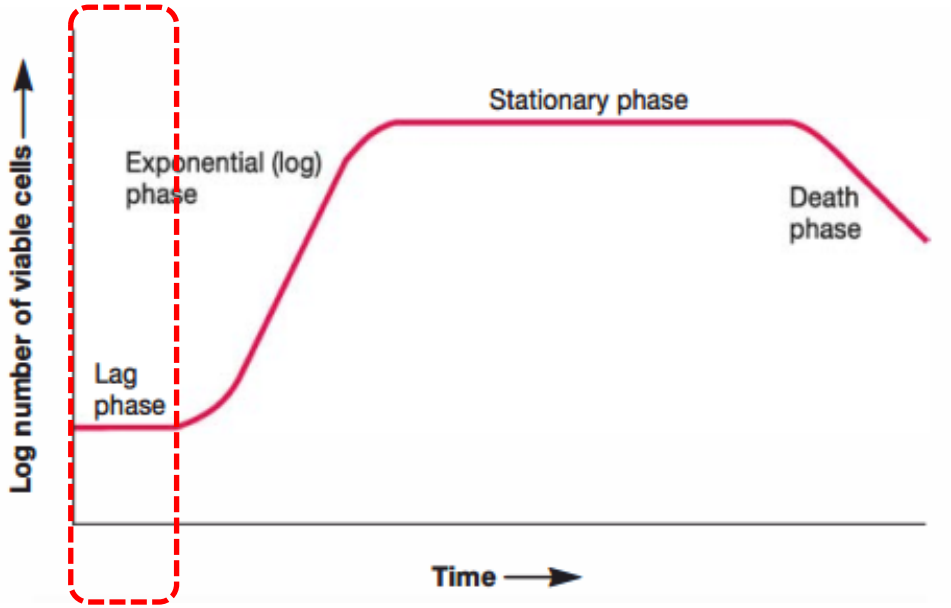
Helmstetter-Cummigs 膜洗脱法





* Zero CFUs only means that too few cells are present to be assayed.

- **2.2.1 Lag phase**
- When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs. This period is called the **lag phase**.



- **Characteristics of lag phase**
- (1) Growth rate constant: R=0;
- (2) Cells become larger or longer;
- (3) RNA content elevated;
- (4) Anabolism blossom;
- (5) Sensitive to NaCl, high-temperature and antibiotics;

- **Factors affecting the length of lag phase**
- (1) Cell age/菌龄
- (2) Inoculum size/接种量

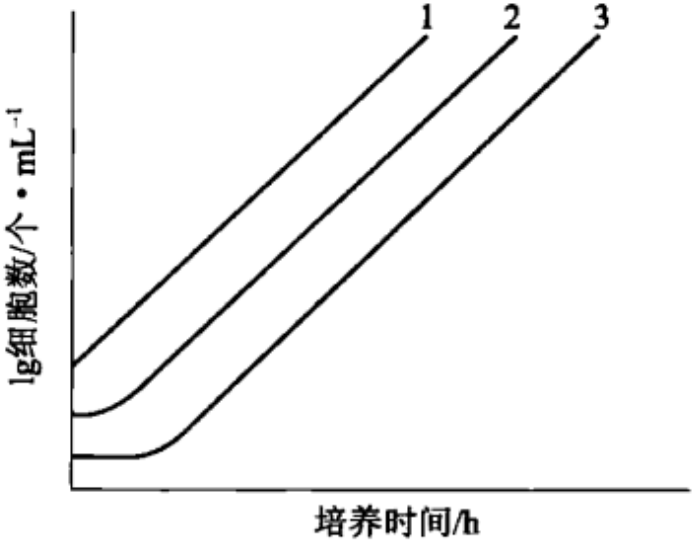
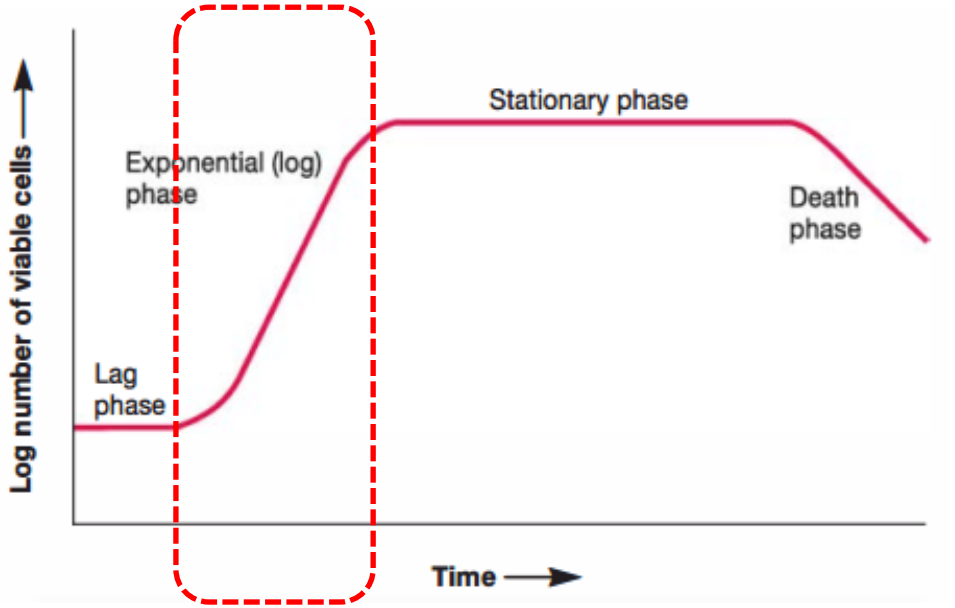


图 6－2　接种量对延滞期的影响

1. 大接种量，2. 中等接种量，3. 小接种量

- (3) Medium component/培养基成分
- (4) Degree of injury/损伤程度

- **2.2.2 Exponential phase**
- At this phase, microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the environmental conditions.



- **Characteristics of the Exp. phase (log phase)**
- (1) R=maximum;
- Generation time

Calculation of the growth rate constant

Let N_0 = the initial population number

N_t = the population at time t

n = the number of generations in time t

For populations reproducing by binary fission

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

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 growth rate constant (k) is the number of generations per unit time $\left(\frac{n}{t}\right)$. Thus

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

Calculation of generation (doubling) time

If a population doubles, then

$$N_t = 2N_0$$

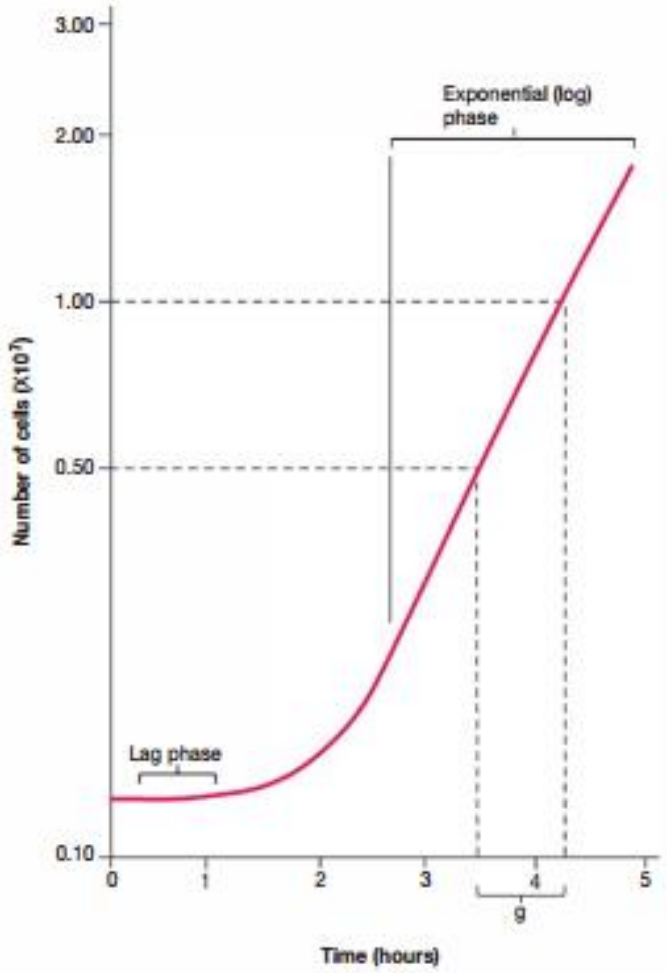
Substitute $2N_0$ into the growth rate constant equation and solve for

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

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

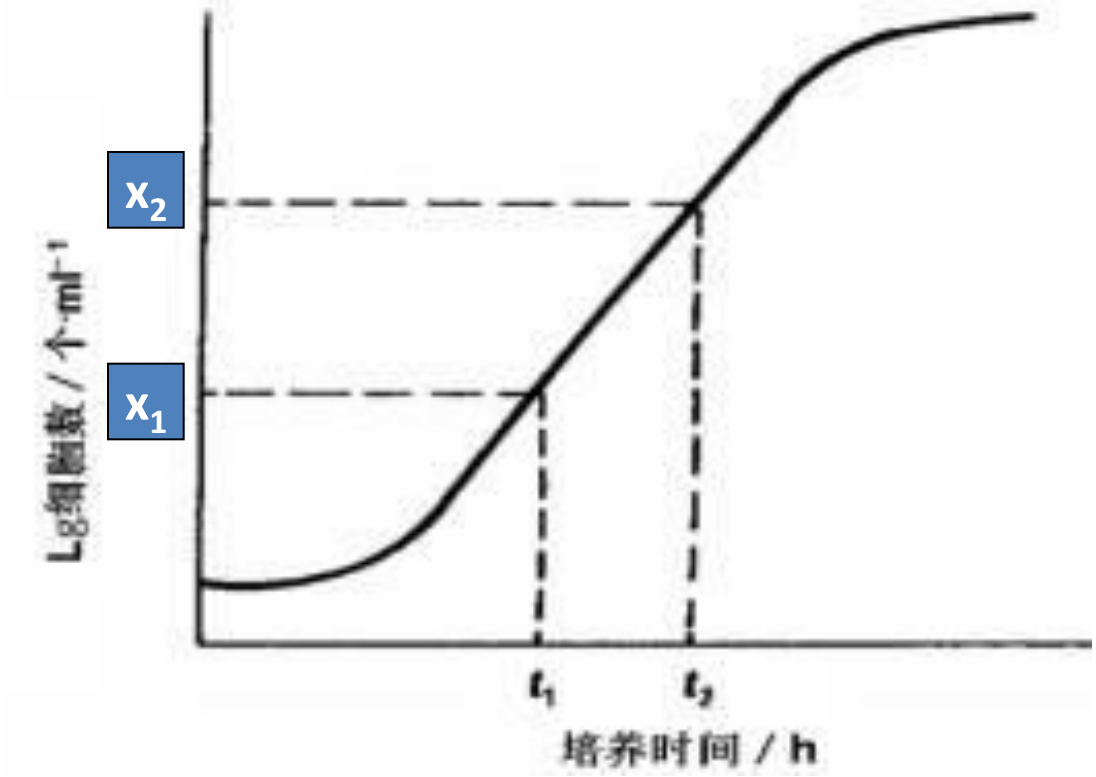
The generation time is the reciprocal of the growth rate constant.

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



- (2) Blanced growth;
- (3) Exuberant metabolism/代谢旺盛;

- **Three important parameters**
- (1) Generations/繁殖代数
- (2) Growth rate constant (R)/生长速率常数
- (3) Generation time/代时



（1）繁殖代数或分裂次数（*n*）

$x_2 = x_1 \cdot 2^n$

$\lg x_2 = \lg x_1 + n\lg 2$

$$n = \frac{\lg x_2 - \lg x_1}{\lg 2} = 3.322(\lg x_2 - \lg x_1)$$

（2）生长速率常数（*R*）

$$R = \frac{n}{t_2 - t_1} = \frac{3.322(\lg x_2 - \lg x_1)}{t_2 - t_1}$$

（3）代时（*G*）

$$G = \frac{1}{R} = \frac{t_2 - t_1}{3.322(\lg x_2 - \lg x_1)}$$

- **Factors affecting the length of Log phase**
- (1) Culture
- (2) Nutrients
- (3) Nutrient concentration
- (4) Temperature

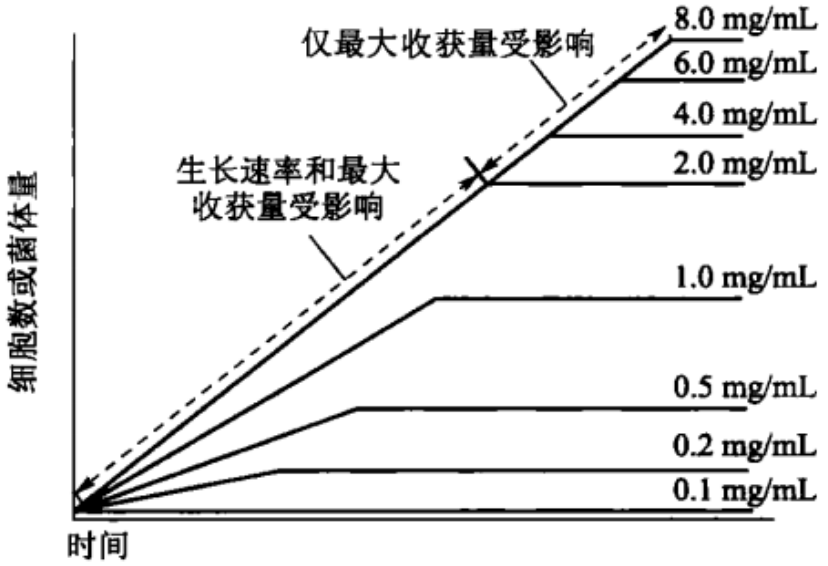
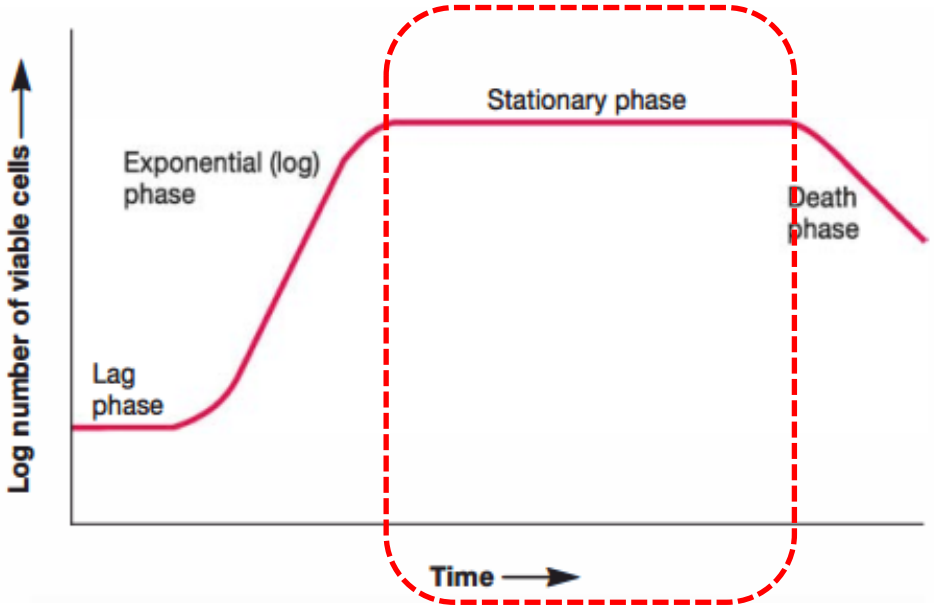


图 6－4　营养物浓度对微生物生长速率和菌体产量的影响

Temp(℃)	G(min)	Temp(℃)	G(min)
10	860	35	22
15	120	40	17.5
20	90	45	20
25	40	47.5	77
30	29		

E. coli

- **2.2.3 Stationary phase**
- In closed system such as batch culture, population growth eventually ceases and the growth curve becomes horizontal.

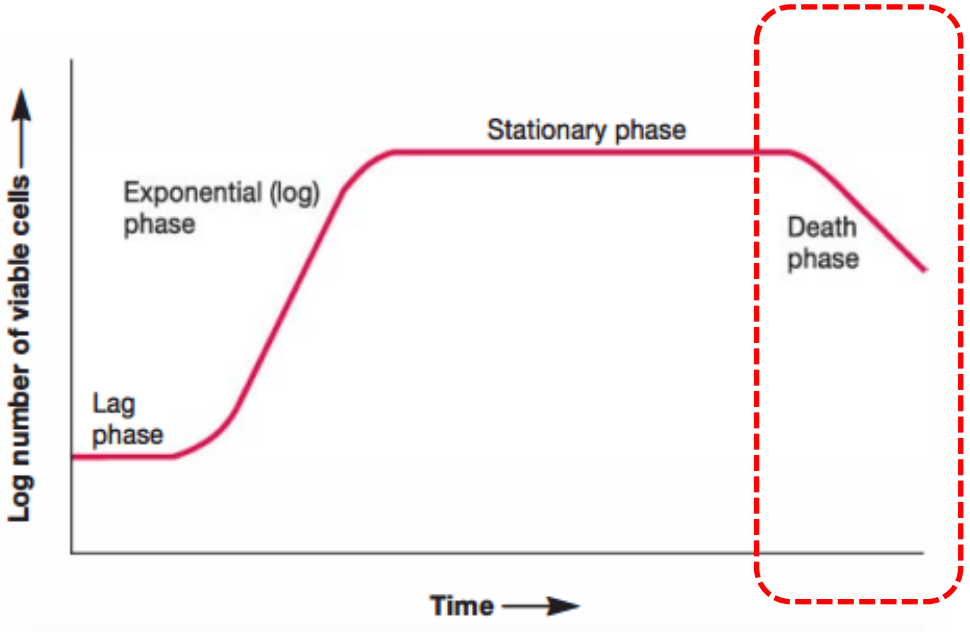


- **Characteristics of stationary phase**
- (1) Highest yield phase;
- Growth yield/生长得率

$$Y = \frac{x - x_0}{C_0 - C} \equiv \frac{x - x_0}{C_0}$$

- (2) R=0;
- (3) Secondary metabolites accumulate;
- (4) Sporulation/芽孢形成

- **2.2.4 Death phase (or decline phase)/衰亡期**
- During this phase, the number of viable cells often declines exponentially, with cells dying at a constant rate.



- **Characteristics of death phase**
- (1) R<0;
- (2) Cell shaped inconformity;
- (3) Secondary metabolites accumulate;
- (4) Spore release;

- **2.3 Microbial continuous culture**
- These systems can maintain a microbial population in exponential growth, growing at a known rate and at a constant biomass concentration for extended periods.
- (1) Turbidostat
- (2) Chemostat

- **2.3.1 Turbidostat**
- The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity.

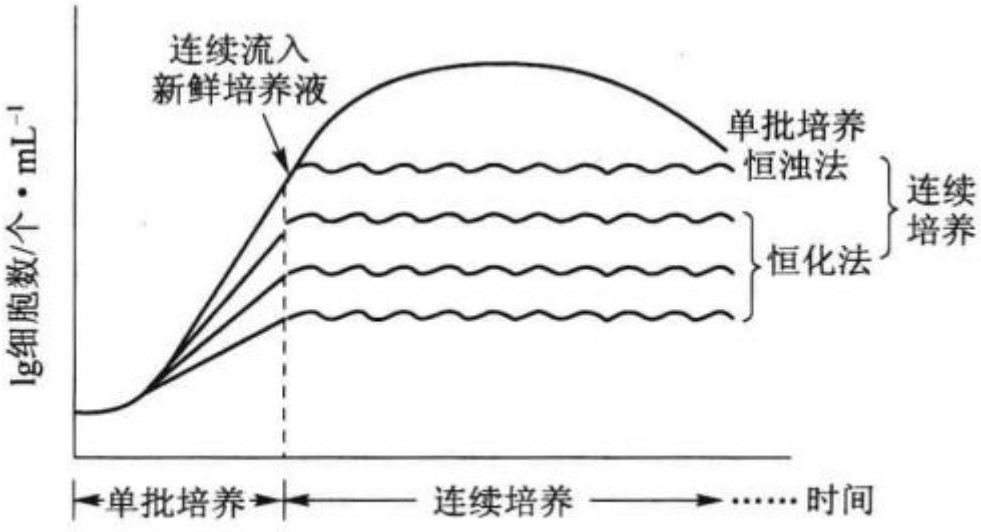
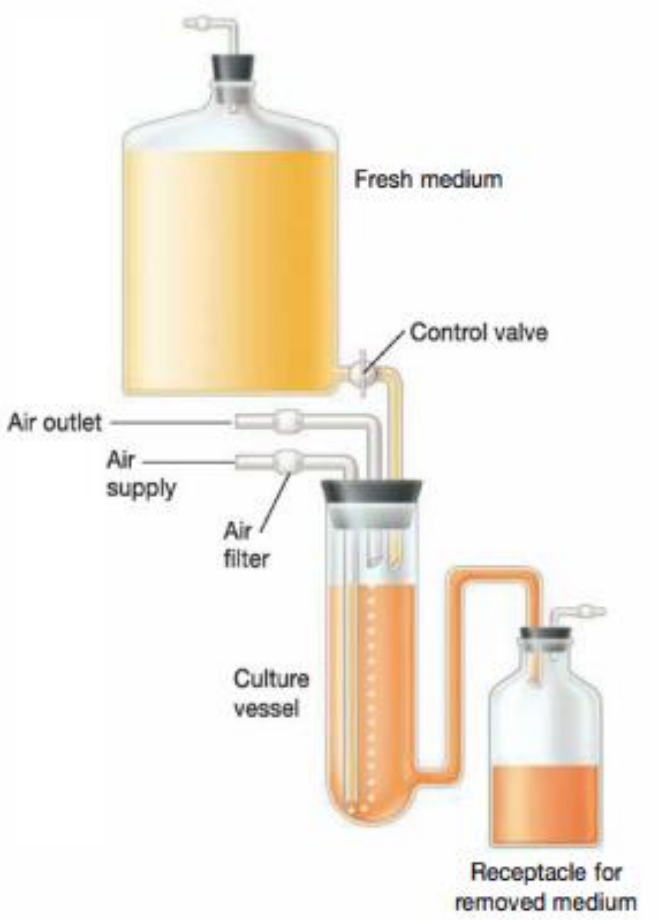


图 6－5　单批培养与连续培养的关系

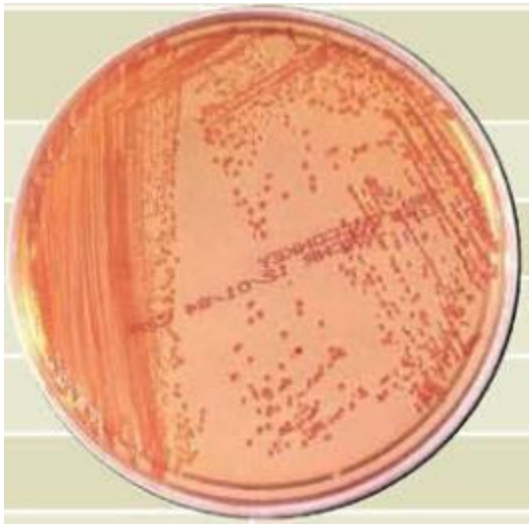
- **2.3.2 Chemostat**
- A chemostat is constructed so that the rate at which a sterile medium is fed into a culture vessel is the same as the rate at which the medium containing microorganisms is removed.



Cultivation of Microbes

- **4.1 Cultivation in laboratory**
- 4.1.1 Solid agar

For aerobic microbe



Agar plate

Slope

For anaerobic microbe

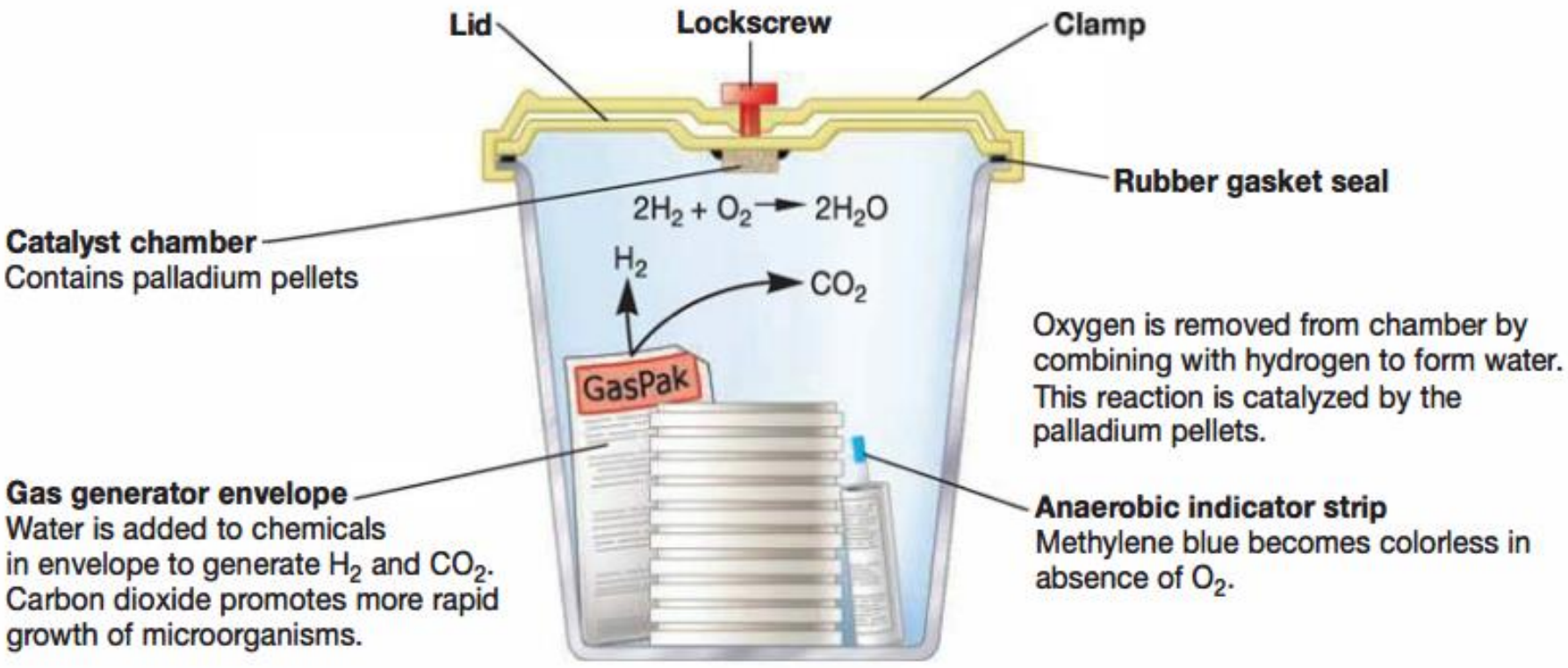
(a)

Anoxic jar

(b)

Anaerobic glove box

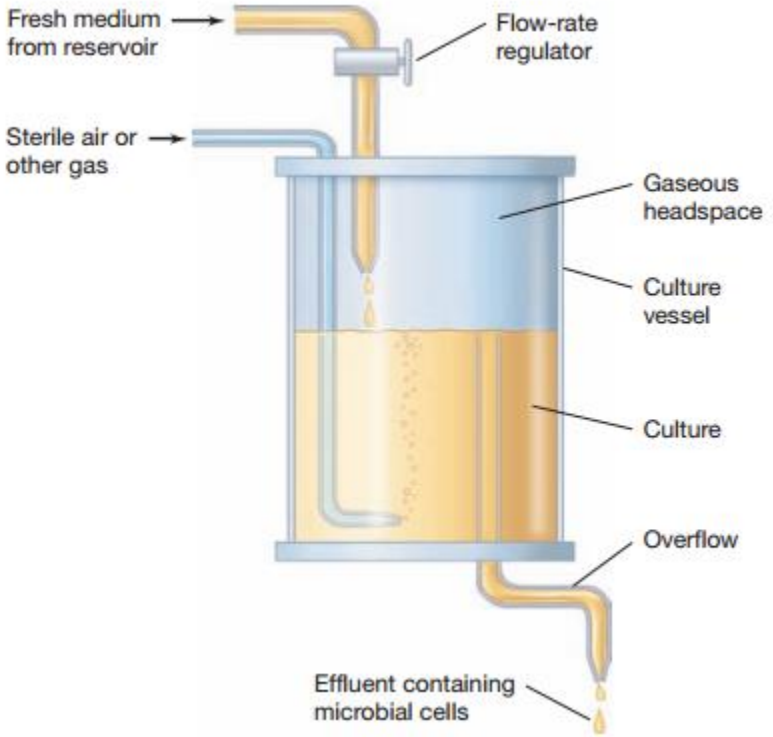
Structure annotation of anaerobic jar



- **4.1.2 Liquid culture**

For aerobic microbes

Shaking flask



Benchtop fermentor

- **4.2 Cultivation in industry**
- 4.2.1 Solid condition

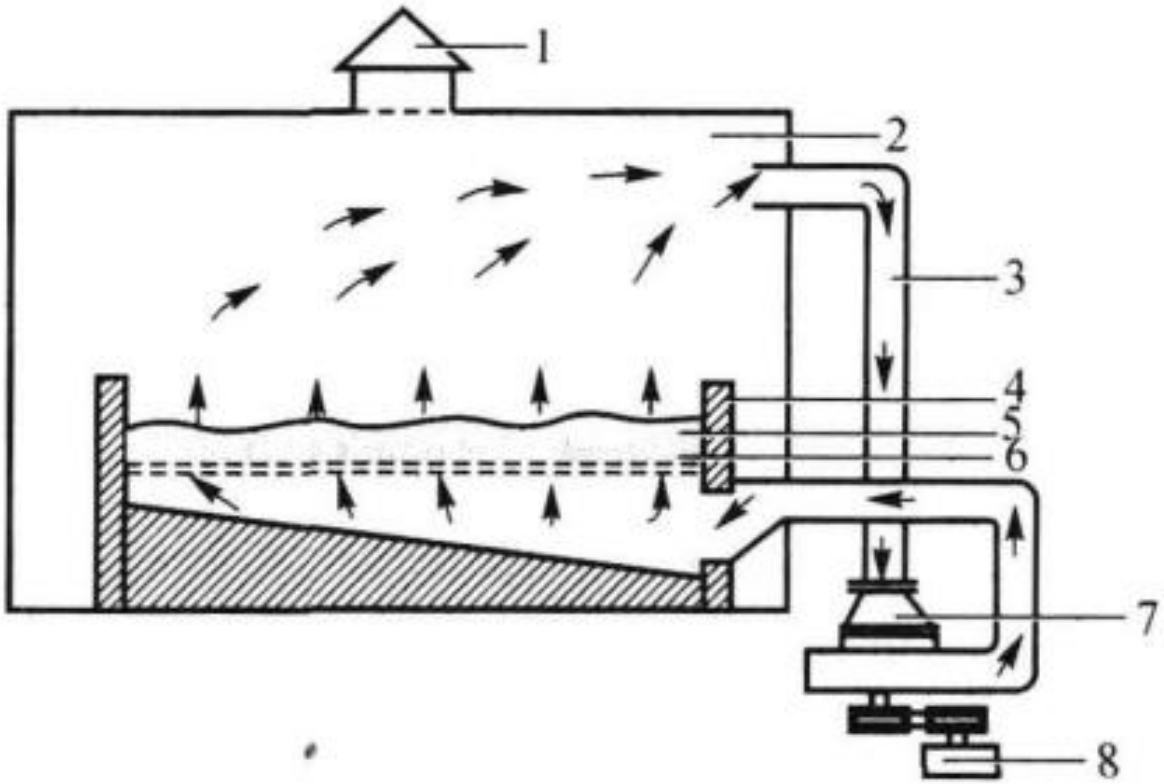
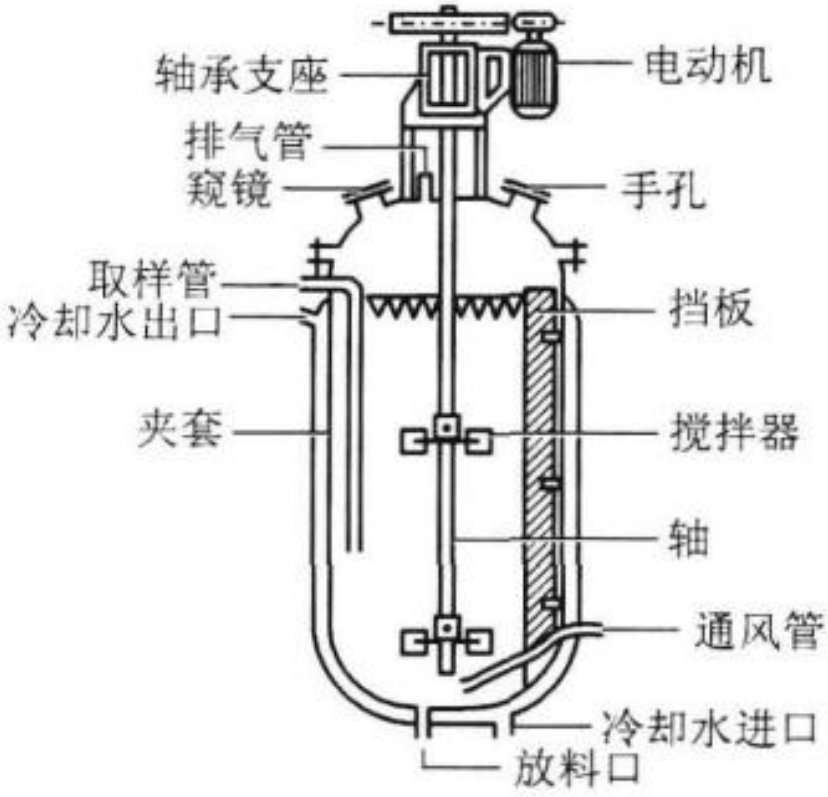


图 6－14　通风曲槽结构模式图

1. 天窗，2. 曲室，3. 风道，4. 曲槽，5. 曲料，
6. 篦架，7. 鼓风机，8. 电动机

- 4.2.2 Liquid condition

Large-scale fermenters

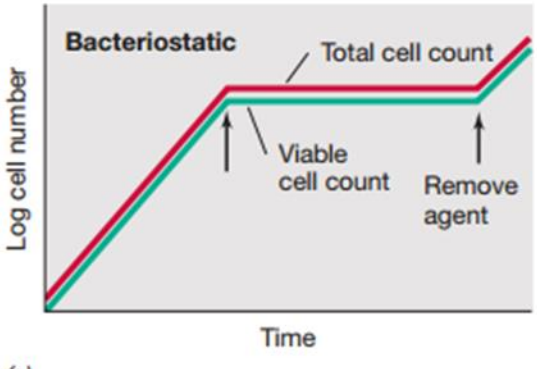


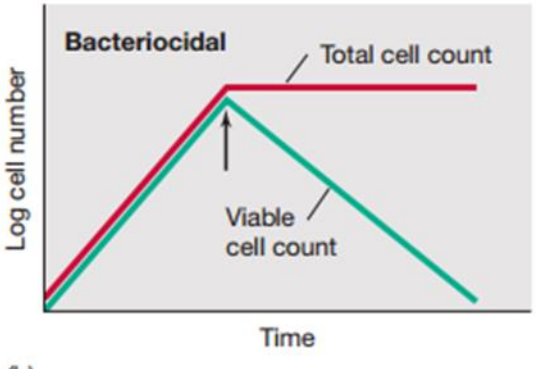
5

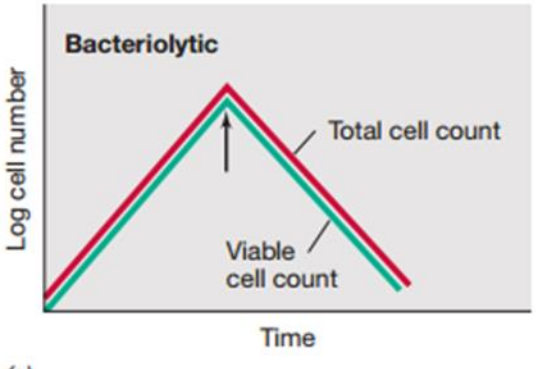
Control the Harmful Microbes

5.1 Four critical conceptions

- **Sterilization:** The complete removal or destruction of all viable microorganisms. Used on inanimate objects.
- **Disinfection:** The destruction or removal of vegetative pathogens but not bacterial endospores
- **Antisepsis:** Chemicals applied to body sources to destroy or inhibit vegetative pathogens.
- **Chemotherapy:** Chemicals used internally to kill or inhibit growth of microorganisms within host tissues.

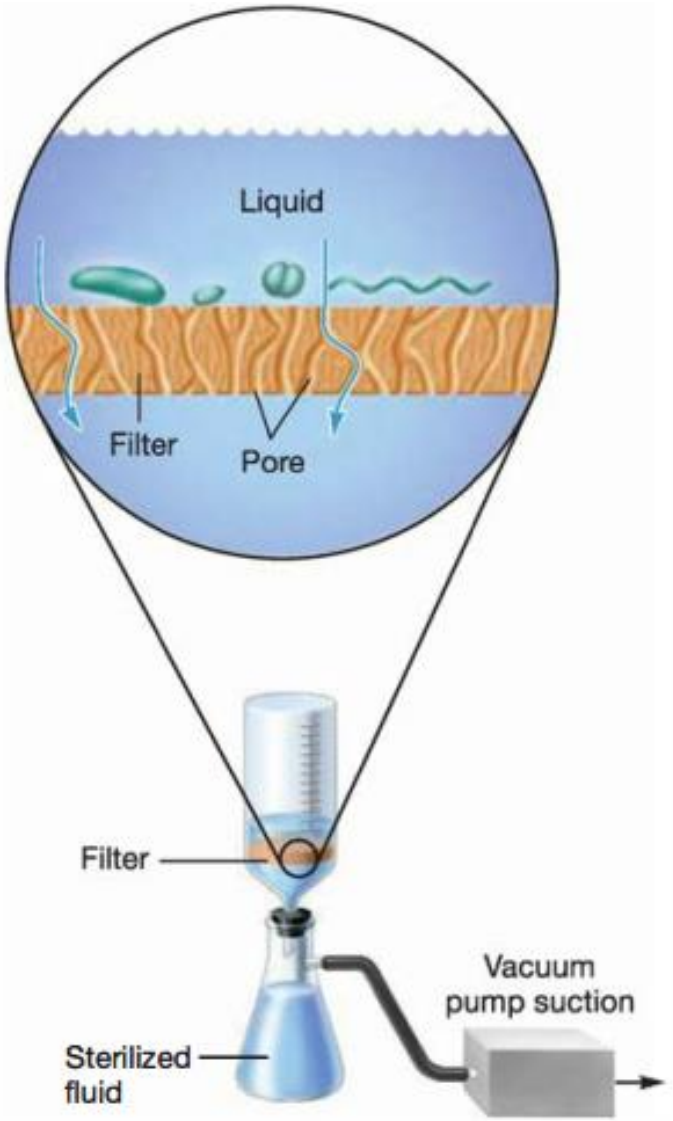


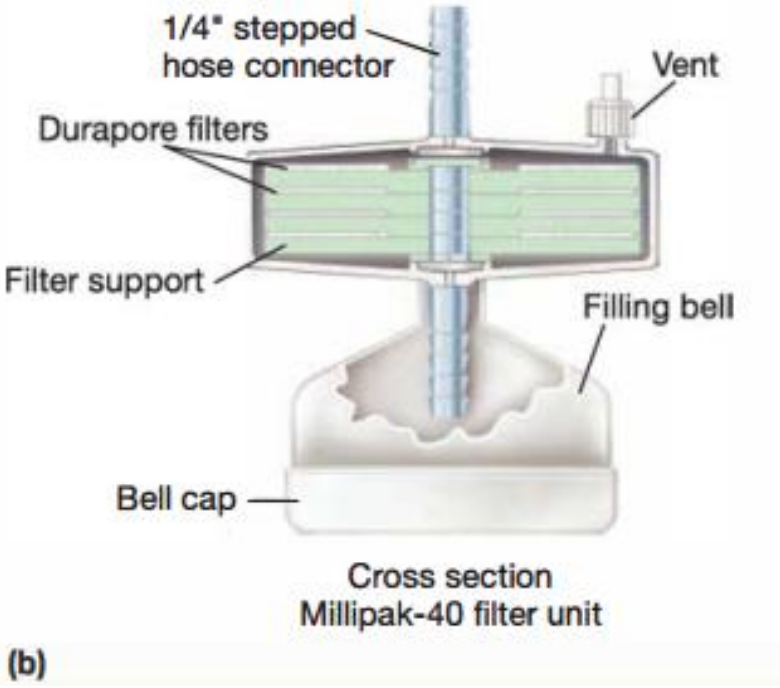


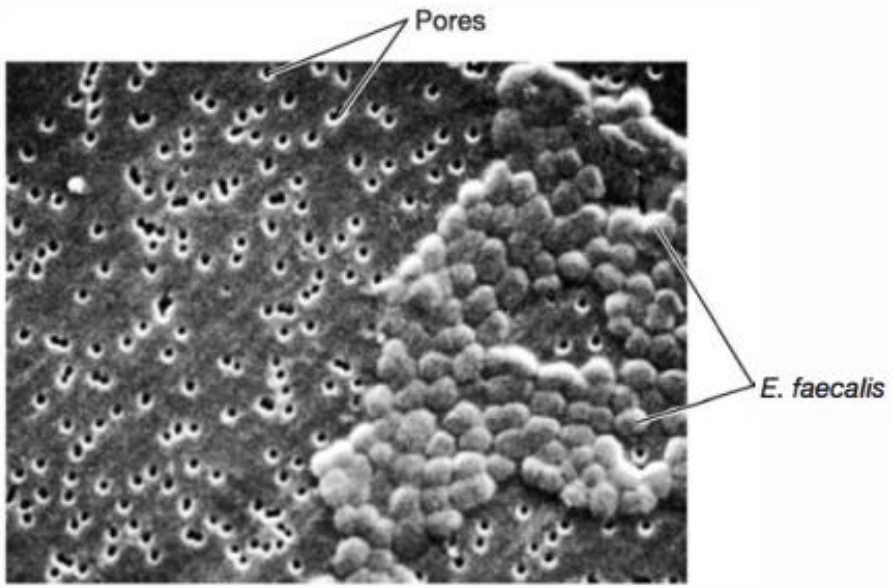


- **5.2 Mechanical removal methods**
- 5.2.1 **Filtration**
- Filtration is an excellent way to reduce the microbial population in solutions of heat-sensitive material and can be used to sterilize various liquids and gases (including air).

- **Membrane filters**

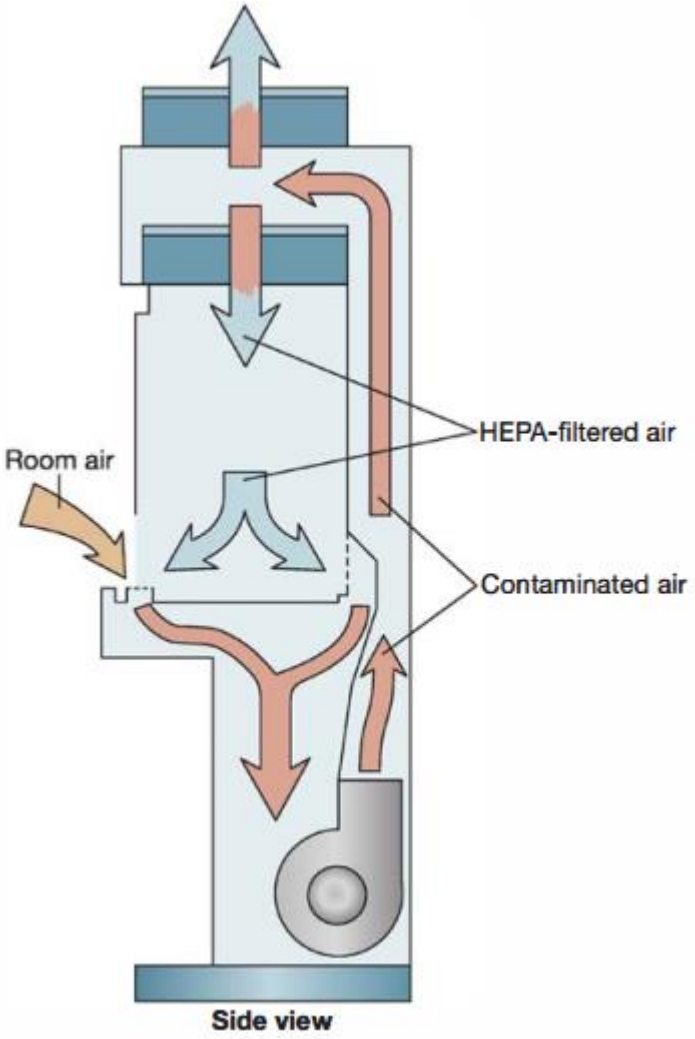




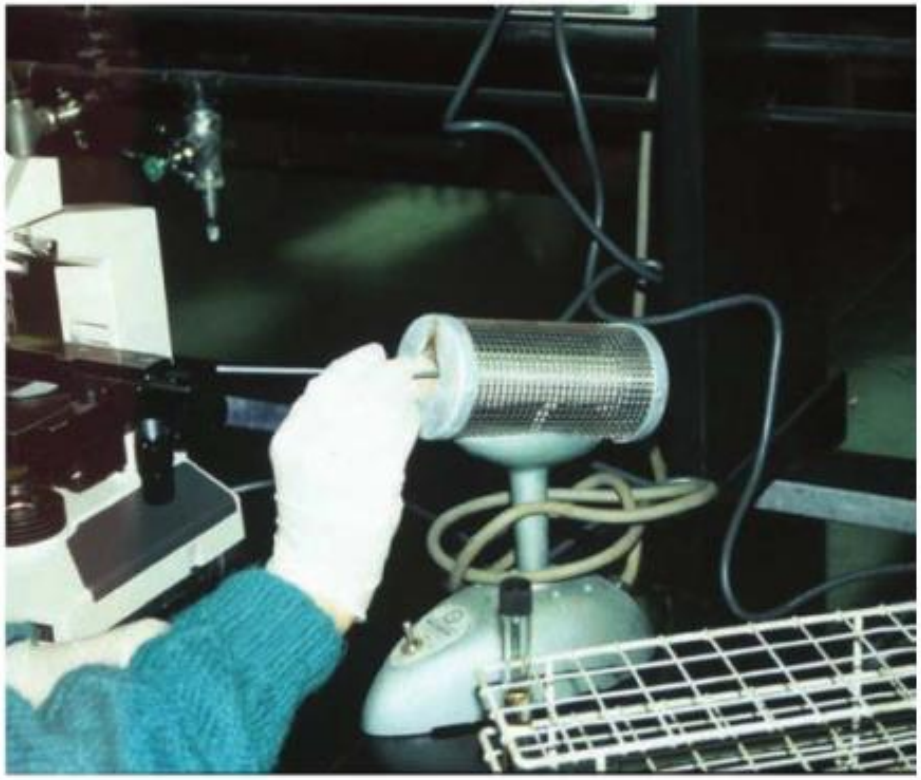


- **Biological safety cabinet**

(a)

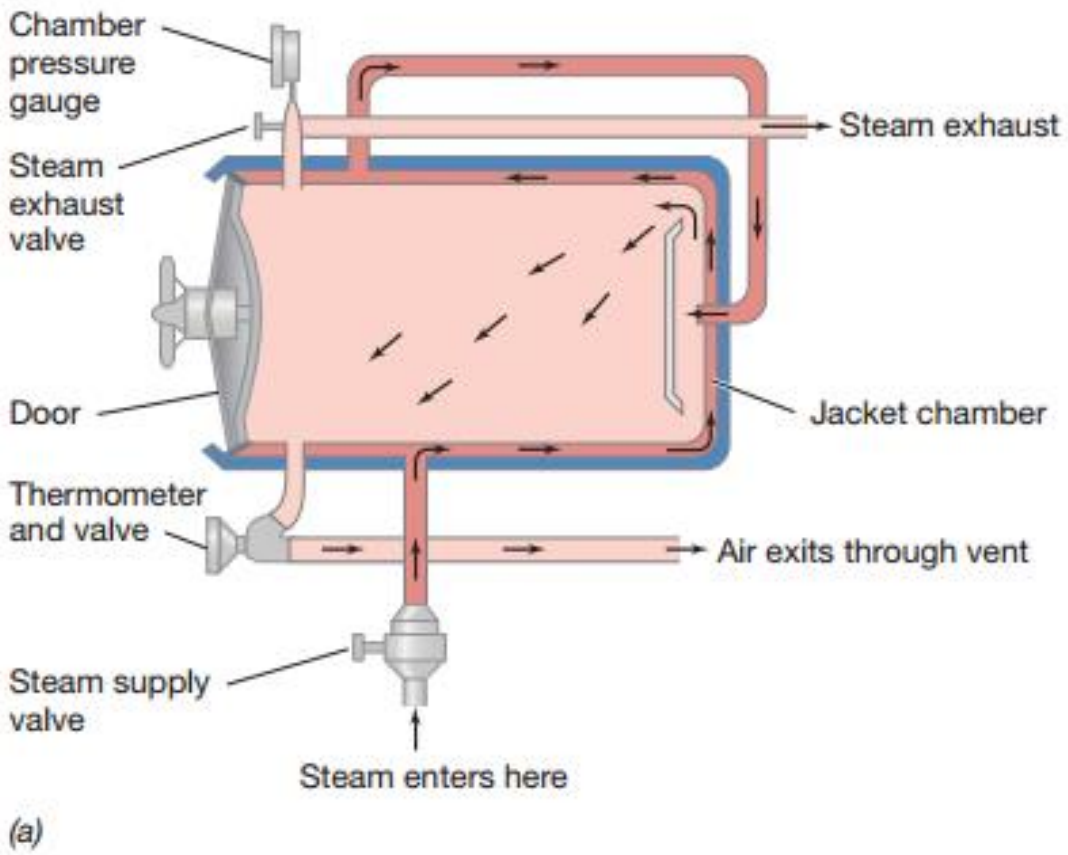


- **5.3 Physical control methods**
- 5.3.1 Dry heat sterilization



- 5.3.2 Pasteurization
- Low temperature holding method; 63^{o}C, 30 min;
- High temperature short time; 72-85^{o}C, 15 s; or 120-140^{o}C, 2-4 s;

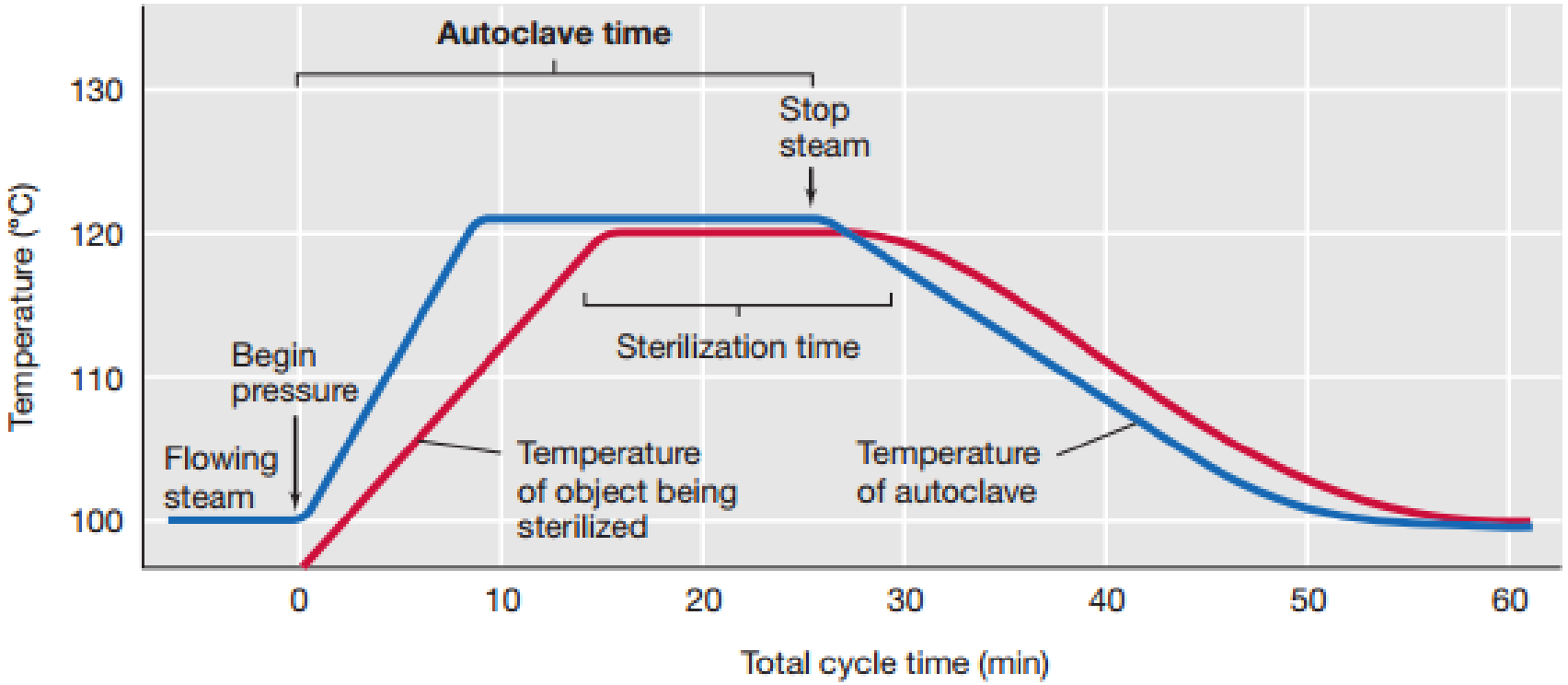
- 5.2.3 Autoclaving



(a)

(c)

- **The autoclave and moist heat sterilization.**

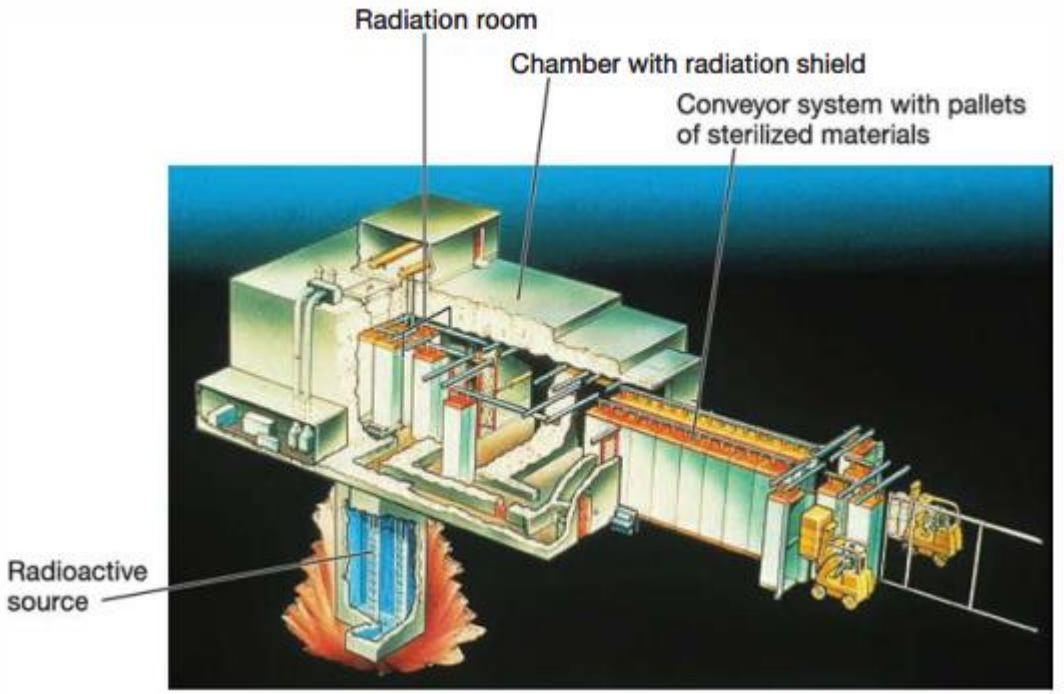


- 5.3.4 Radiation

  UV radiation causes thymine-thymine dimerization of DNA, preventing replication and transcription.

- 5.3.5 Ionizing radiation

  It is an excellent sterilizing agent and penetrates deep into objects.



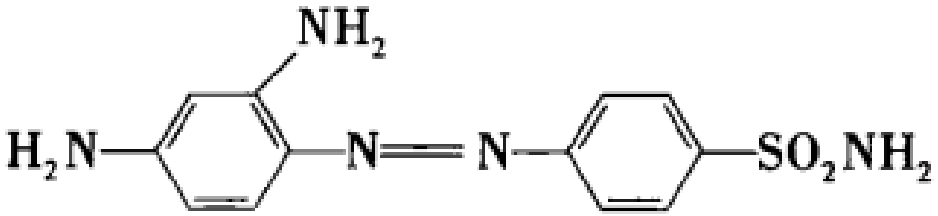
- 5.4 Chemical Control Agents
  - Physical agents are generally used to sterilize objects. Chemicals, on the other hand, are more often employed in disinfection and antisepsis.

  - 5.4.1 Surface disinfectants
  - 5.4.2 Antiseptics

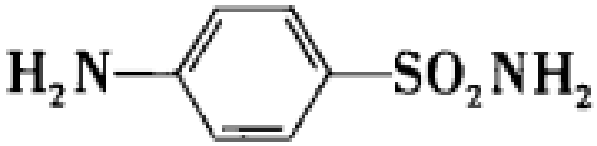
Table 5.7 Antiseptics, sterilants, disinfectants, and sanitizers[a]

Agent	*Mode of action*	*Use*
Antiseptics (germicides)		
Alcohol (60–85% ethanol or isopropanol in water)	Lipid solvent and protein denaturant	Topical antiseptic
Phenol-containing compounds (hexachlorophene, triclosan, chloroxylenol, chlorhexidine)	Disrupts cytoplasmic membrane	Soaps, lotions, cosmetics, deodorants, topical disinfectants; paper, leather, and textile industries
Cationic detergents, especially quaternary ammonium compounds (benzalkonium chloride)	Disrupts cytoplasmic membrane	Soaps, lotions, topical disinfectants; metal and petroleum industries
Hydrogen peroxide (3% solution)	Oxidizing agent	Topical antiseptic
Iodophors (Betadine®)	Iodinates proteins, rendering them nonfunctional; oxidizing agent	Topical antiseptic
Octenidine	Cationic surfactant, disrupts cytoplasmic membrane	Topical antiseptic
Sterilants, disinfectants, and sanitizers		
Alcohol (60–85% ethanol or isopropanol in water)	Lipid solvent and protein denaturant	General purpose disinfectant for virtually any surface
Cationic detergents (quaternary ammonium compounds, Lysol® and many related disinfectants)	Interact with phospholipids	Disinfectant/sanitizer for medical instruments, food and dairy equipment
Chlorine gas	Oxidizing agent	Disinfectant for drinking water and electrical/nuclear cooling towers
Chlorine compounds (chloramines, sodium hypochlorite, sodium chlorite, chlorine dioxide)	Oxidizing agent	Disinfectant/sanitizer for medical instruments, food/dairy equipment, and in water purification
Copper sulfate	Protein precipitant	Algicide in swimming pools
Ethylene oxide (gas)	Alkylating agent	Sterilant for temperature-sensitive materials such as plastics

- 5.4.3 Chemotherapeutant
- (1) Antimetabolite-Sulphonamide



Biological process



Sulphonamide

analogue

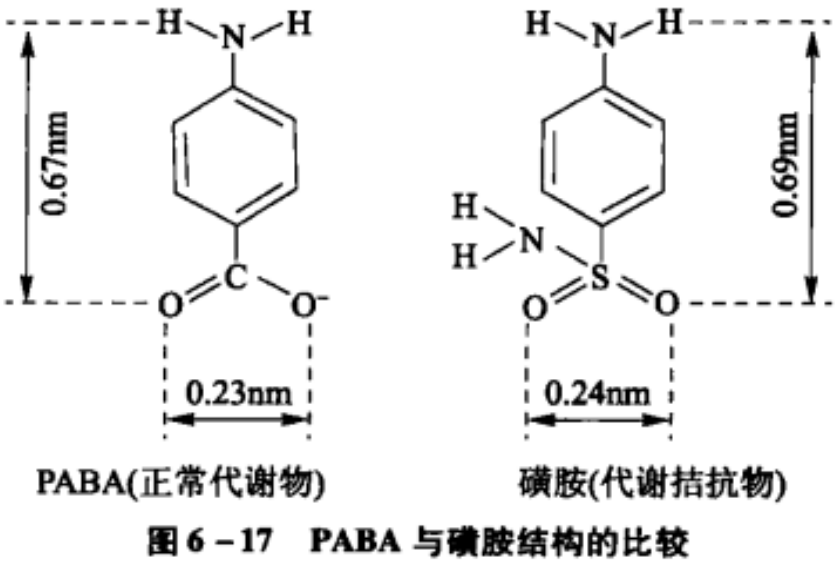
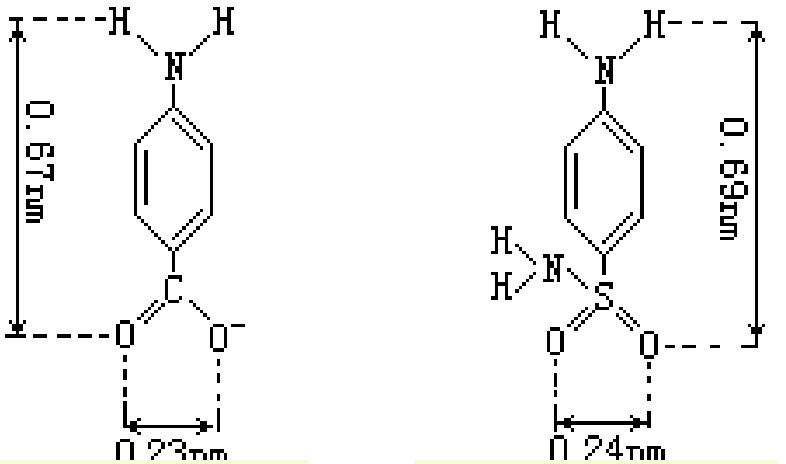
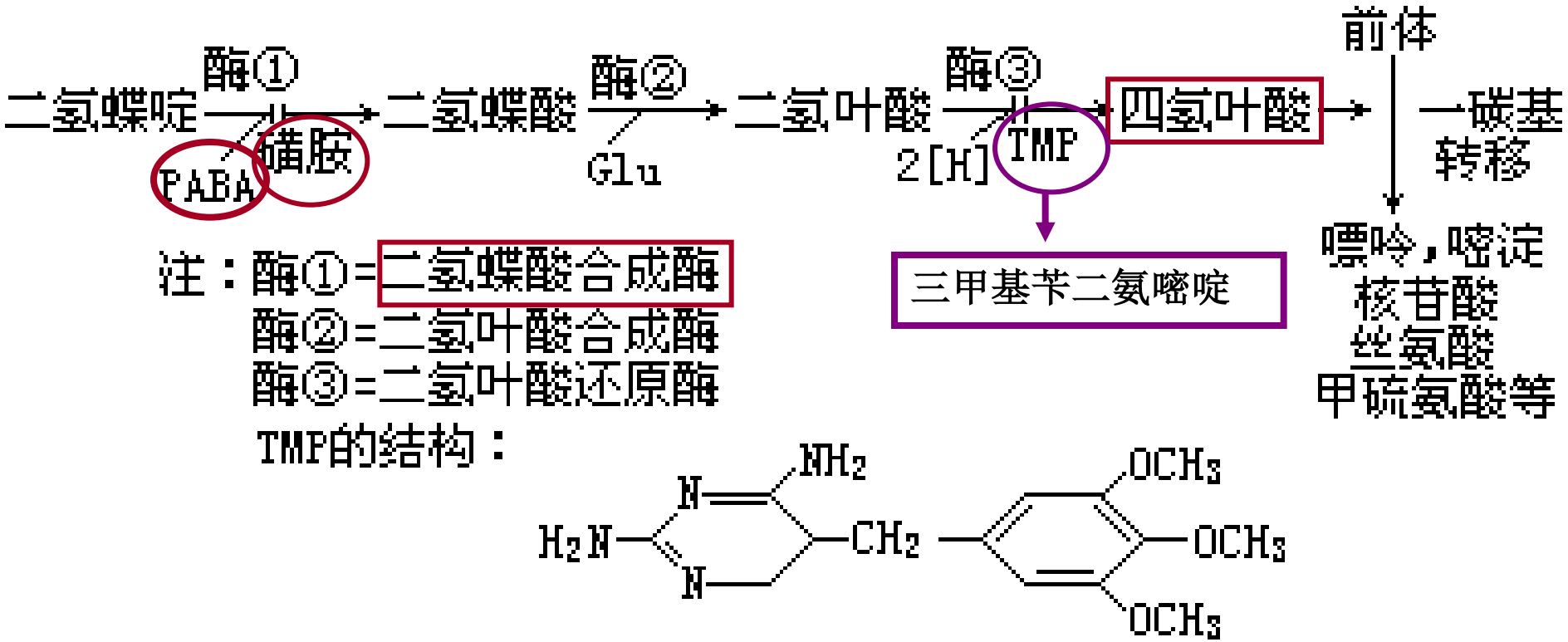


图 6－17　PABA 与磺胺结构的比较

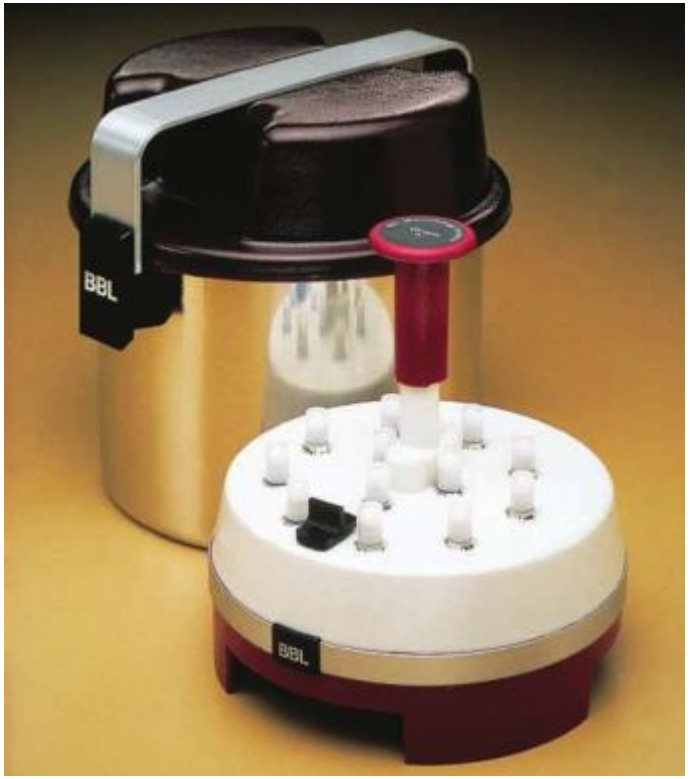


PABA（对氨基苯甲酸）为细菌必需的生长因子

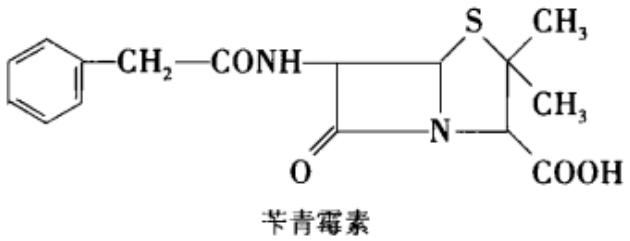
图 7-19 PABA与磺胺结构的比较



- A successful chemotherapeutic agent has selective toxicity: it kills or inhibits the microbial pathogen while damaging the host as little as possible.



- (2) Antibiotics-Penicillin



苄青霉素

ampicillin

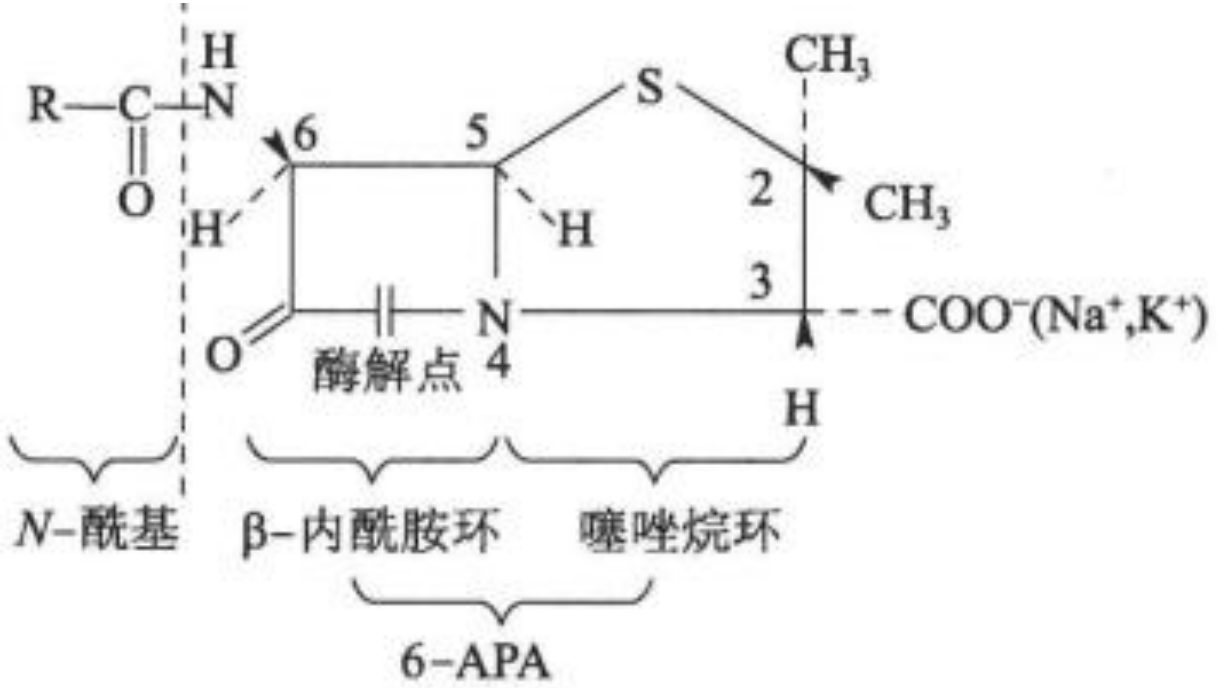


图 6－20　青霉素的核心结构

EXERCISE

1. **Describe how an autoclave works. What conditions are required for sterilization by moist heat? What three things must one do when operating an autoclave to help ensure success?**

2. In the past, spoiled milk was responsible for a significant proportion of infant deaths. Why is untreated milk easily spoiled?

- **2. Define the following terms: sterilization, sterilant, disinfection, disinfectant, sanitization, antisepsis, antiseptic, chemotherapy, biocide.**

- **3. What is the difference between bactericidal and bacteriostatic? To which category do you think most household cleaners belong? Why?**