

## **Chapter 8 Microbial growth and control**

### **Section 1 Concept of microbial growth**

#### 1 Definition

Individual growth of microbes is defined as an increase in cellular weight, volume or size. This will result in an increase in the number of cells at certain time, which is called reproduction of individual cell. And reproduction will result in growth of microbial population.

**In most case, microbial growth is defined as the growth of microbial population.**

#### 2 Individual reproduction of procaryotes

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

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

#### 3 Individual reproduction of eucaryotes

There are 4 stages in eukaryotic cell, they are G1 stage, S stage, G2 stage and M stage.

There are 3 stages in prokaryotic cell, they are lstage, R stage and D stage.

### **Section 2 Measurement of microbial growth**

The methods to measure microbial growth are different from those for plant or animal growth.

#### 1 Measurement of number of cells

##### 1) Total count

Total number of microbes can be measured by direct count with microscope and determination by turbidities (OD) .

##### 2) Viable count

Number of the viable microbes can be measured by plate count and Most Probable Number Method.

#### 2 Measurement of biomass

Biomass of microbes can be measured by dry weight of cell, quantity of protein(or DNA, ATP) and metabolic activity.

### **Section 3 Growth curve of bacteria**

When microorganisms are grown in a closed system, population growth remains exponential for only a few generations and then enters a stationary phase due to factors like nutrient limitation and waste accumulation. If a population is cultured in an open system with continual nutrient addition and waste removal, the exponential phase can be maintained for long periods

Growth curve is used to describe the growth of bacteria in a closed system.

#### 1 Growth curve of bacteria

In Lag Phase, cells begin to synthesize inducible enzymes and use stored food reserves.

In Exponential Phase, the cells multiply rapidly and the rate of multiplication is constant.

In Stationary Phase, death rate is equal to rate of increase.

In Death Phase, cells begin to die at a more rapid rate than that of reproduction.

## 2 Continuous culture

In batch culture or closed culture, culture medium is filled only once and the mixture of culture is removed from the container after the fermentation.

In Continuous Culture, with adding of nutrient and removing of metabolites, exponential period lasts for any long time. It is also called open culture.

In Turbidostat, the rate at which new medium enters the growth chamber is controlled according to the cell density.

In Chemostat, the 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.

In Synchronous Culture, all the cells are in the similar physiological condition.

## **Section 4 Factors that affect the growth of microbes**

Except for nutrients, there are other factors that have impacts on the growth or survival of microbes.

Temperature, oxygen concentration, water availability, pH, pressure, radiation, and a number of other environmental factors influence microbial growth.

Yet many microorganisms, and particularly bacteria, have managed to adapt and flourish under environmental extremes that would destroy most organisms.

### 1 Temperature

Three cardinal points of temperature are the minimum growth temperature, the maximum growth temperature and the optimum growth temperature. Microbes 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.

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

Effect of extreme temperature on microorganisms:

Under extremely low temperature, microbes may stop growing, but they may enter a state of dormancy and will restore when the temperature is normal. For example, food and strains of microbes with excellent properties are stored under high temperature.

Under extremely high temperature, microbes are killed or sterilized. Therefore, sterilization is carried out under extremely high temperature.

### 2 Oxygen

Aerobes are those microbes which can grow where oxygen presents. They include Obligate aerobes/Facultative aerobes/Microaerophiles.

Anaerobes are those microbes which can not grow where oxygen presents. They include Obligate anaerobes/ Aerotolerant anaerobes.

a, Obligate aerobes grow only in the short distance to which the oxygen diffuses into the medium.

b, Facultative anaerobes grow best near the surface, where oxygen is available, but occur throughout the tube.

e, Microaerophiles do not tolerate high atmospheric concentrations of oxygen and their growth occurs only in a narrow band of optimal oxygen concentration.

d, Aerotolerant anaerobes grow evenly throughout the tube but is not better at the surface because the organisms do not use oxygen.

e, Obligate anaerobes are sensitive to oxygen, and there is no growth near the surface.

Eh more than +0.3 is favorable for Aerobe.

Eh more than +0.1 is favorable for aerobic Respiration of Facultative anaerobes.

Eh less than +0.1 is favorable for fermentation of Facultative anaerobes.

Eh less than -0.1 is favorable for anaerobes.

### 3 pH value

Neutral condition with pH 6.5-7.5 is favorable for bacteria, algae and protozoa.

Acidic condition with pH 5.0-6.0 is favorable for fungi.

Alkaline condition with pH 7.8-8.0 is favorable for actinomycetes.

### 4 Concentration of media and humidity

#### 1) Concentration and water activity

The water activity of a solution is the ratio of the solution's vapor pressure to that of pure water.

When the concentration of media increases, the water activity of the solution decreases.

#### 2) Concentration and osmotic pressure

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.

When the concentration of media increases the osmotic pressure of the solution decreases

#### 3) humidity and microbial growth

Humidity means the relative humidity of the air around the media.

Humidity is often related to the aerial growth of fungus. For examples, the growth of mould and mushroom is greatly affected by humidity.

### 5 Radiation

Visible light is not essential to most of microbe but is needed by the photosynthetic microbes.

Ultraviolet,  $\alpha$ ,  $\beta$ ,  $\gamma$ , X ray are harmful to most of microbes, however, they can be used in breeding of microbes.

## Section 5 Culture of microbes

### 1 In lab

1) In solid media

Aerobes should be cultured in test-tube slant, agar plate, kolle flask.

Anaerobes should be cultured in Hungate roll-tube ,anaerobic jar, anaerobic glove box or medium supplemented by resazurin.

2) In liquid media

Aerobes should be cultured in tube culture, shake-flask and benchtop fermenter.

Anaerobes should be cultured in medium supplemented by vitamin C.

2 In practice

1) In solid media

Aerobes should be cultured in mouldy bran, culture on plates and shelf.

Anaerobes should be cultured in production of liquor.

2) In liquid media

Aerobes should be cultured in shallow pan cultivation, fermenter, immobilized cell.

Anaerobes should be cultured in anaerobic fermenters.

## Section 6 Control of microbes

1 Concepts

Sterilization is the process of destroying all forms of microbial life on an object or in a material, also called bacteriocidation, bacteriolysis.

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

Antisepsis is the process of inhibiting the growth of decay-causing microbes in food and others.

Chemotherapy is the process of inhibiting the growth of pathogen in animal.

2 Sterilization by high temperature

1) Types of sterilization by high temperature

Dry heat sterilization should be conducted under 165 °C for 2h, which is also called incineration or combustion.

Moist heat sterilization is conducted with moisture.

Under normal air pressure, moist heat sterilization includes Pasteurization, low temperature holding(LTH 63 °C, 30min), high temperature short time(HTST72 °C, 15s), Boiling, Fractional sterilization, or tyndallization.

Under extra air pressure it includes Normal autoclaving and Continuous autoclaving

3 Other means of sterilization

Radiation is conducted under X-ray, untraviolet, and so on.

Filtration is conducted by passing the solution through certain kind of material which allows only smaller particle to go through.

4 Chemical bactericide, disinfectant and chemotherapeutant

1) Surface disinfectant

Surface disinfectants include Phenol/phenolics, Alcohols, Halogens, F, Cl, Br, I, Heavy metals, Cu, Hg, Oxidant, Acid, alkylating agents, Staining agent.

2) Antimetabolite

Antimetabolites include Metabolite analogue (sulphonamides, sulfa drugs).....

3) Antibiotics

4) Semi-synthetic antibiotics and biopharmaceutin