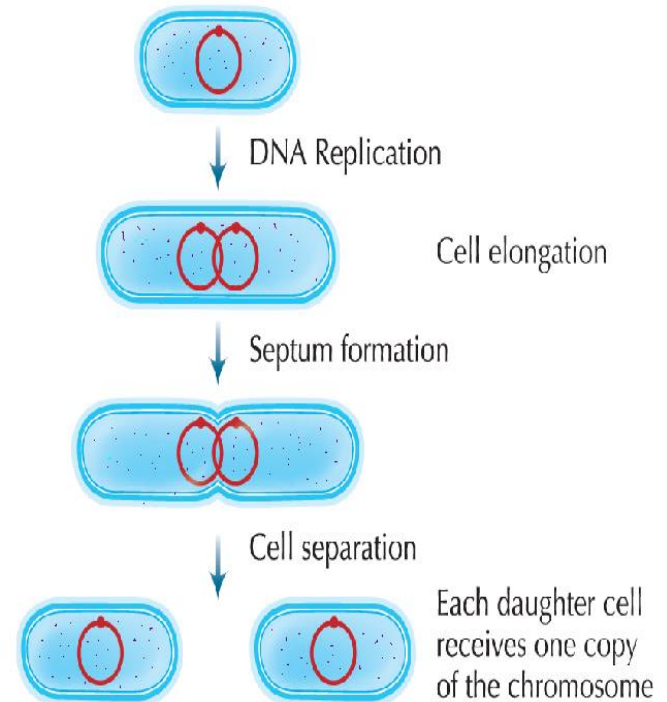


# **Microbial Growth**

- Microbial growth refers to increase in number of cells, not the size
- Requirements chemical and physical
- Chemical requirements include water, carbon, nitrogen, minerals, oxygen, organic growth factors
- Physical aspects include temperature, pH and osmotic pressure

- Bacteria normally reproduce by binary fission
- First step in division cell elongation, duplication of the chromosomal DNA
- Cell wall and cell membrane then begin to grow inward from all sides at a point
- Ingrowing cell walls meet forming a cross wall and two individual cells are formed
- Few bacterial species reproduce by budding
- Filamentous bacteria (*Actinomycetes*) reproduce by producing spores or by fragmentation



BINARY FISSION IN BACTERIA

# Generation Time

- Bacterial numbers increases exponentially
- time required for a cell to divide is called the **generation time or doubling time**
- The increase in population is always  $2^n$  where n is the number of generations
- ∴ After 6 generations the number of cells will be:
- Population =  $2^6 = 64$
- After 20 generations =  $2^{20} = 1,048,576$
- This can be expressed as  $N_t = N_0 \times 2^n$
- $N_t$  = the population at time 't'
- $N_0$  = the initial number
- n = number of generation in time 't'

# The growth Phases

- Microorganisms are cultured in a batch culture or closed system
- Growth of microorganisms can be plotted as the logarithm of cell number versus the incubation time
- Growth curve has four distinct phases

## **1. Lag Phase**

- No immediate increase in cell number occurs
- Lag phase is also called tooling up phase
- Lag phase varies considerably in length depending upon factors like: Nature of the medium, condition of microorganisms

## **2. Exponential phase**

- microorganisms grow and divide at rapid rate
- rate of growth is constant
- population is uniform in terms of chemical and physiological properties

## **3. Stationary phase**

- Population growth ceases and growth curve becomes horizontal
- attained by bacteria at a population density of around  $10^9$  cells per ml

- Microbial populations enter the stationary phase due to nutrient limitation ,Depletion of oxygen and Production of toxic metabolites

#### **4. Declining phase (or) Death Phase:**

- Changes like nutrient depletion, build up of toxic waste lead to decline in the number of viable cells
- death of the population is usually logarithmic similar to its growth

# Measurement of cell growth

- **Direct microscopic count (DMC)**
- Petroff – Hausser counting chamber
- Haemocytometer can be used for larger eukaryotic microorganisms
- Number of microorganisms calculated by taking into account the chamber's volume and the dilution of the sample
- 25 squares covering an area of  $1 \text{ mm}^2$
- Area of  $1 \text{ mm}^2$  is calculated / counted
- Chamber is  $0.02 \text{ mm}$  deep



- Bacteria / mm<sup>3</sup> = number / square x 25 squares x 50
- Number of bacteria per cm<sup>2</sup> is 1000 times this value.
- Disadvantages:
  - small volume is sampled
  - not possible to distinguish live and dead cells

# Plating techniques

- Mixture of cells is diluted and spread out on an agar surface with agar medium in a petridish, cell grows into completely separate colony
- Spread plate
- Pour plate
- Spread plating, 0.1 or 0.5 ml of diluted samples are spread out on an agar surface so that every cell grows into a colony
- Pour plating, the original sample is diluted several times, (1 ml) of diluted samples are mixed with liquid agar
- Each cell is fixed in place, forms an individual colony
- Results of plating technique are always expressed as colony forming units

# Membrane Filtration Technique

- Sample is drawn through a special membrane filter
- Filter is then placed on an agar medium, incubated until each cell forms a separate colony

# Indirect methods

- **Measurement of cell mass**
- **Turbidimetry**
- microbial cells scatter light striking them
- amount of scattering is proportional to the concentration of cells present