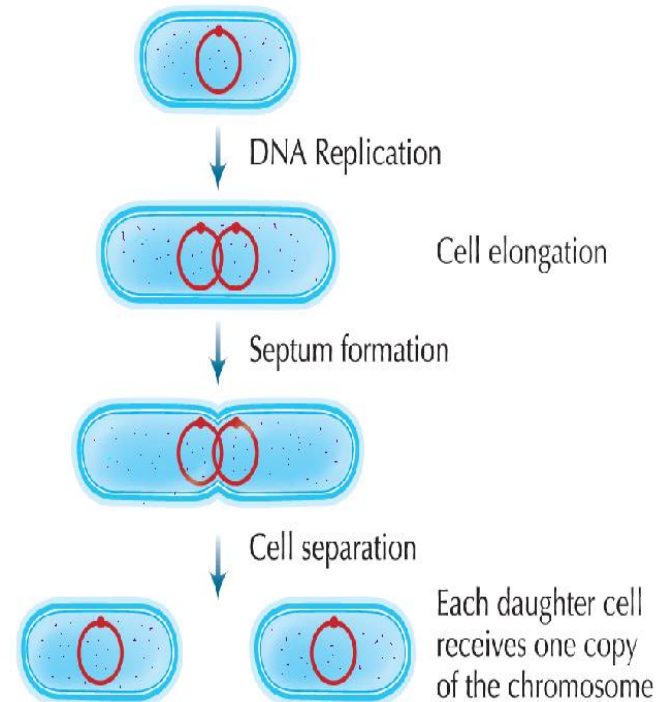


Growth of Bacterial Culture & Culture Media

- 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 (*Actinomyces*) 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
- \therefore 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 Curve

- 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
- **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

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

Continuous culture of microorganisms

- microbial population can be maintained at a constant biomass concentration for extended periods
- types of continuous culture systems
chemostats, turbidostats
- Chemostat, fresh medium contains a limiting amount of an essential nutrient
- Growth rate determined by the rate of flow of medium

- **The Turbidostat**
- a photocell that measures the absorbance or turbidity of the culture
- flow rate of media is automatically regulated maintain a predetermined turbidity or cell density

Synchronous culture

- Synchronous culture cells which are at the same stage of cell cycle
- Synchrony in bacteria is accomplished repetitive shifts temperature fresh nutrients to cultures
- Helmstetter – Cummings technique

Culture Media

- **Synthetic / Defined media**
- defined medium or synthetic medium
- **Complex media**
- Media that contain ingredients of unknown chemical composition
- (TSA), (NA), (PCA)
- general purpose media or non selective media

- **Selective media**
- Favour growth of particular group of microorganisms. Specific salts / dyes are used to suppress organisms other than the target (Bile salts - inhibiting G+ve bacteria)
- Examples - BSA, BPA
- **Differential media**
- Media that distinguish between different groups of bacteria permit tentative identification of microorganisms

Quantification of microorganisms

- **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 mm^2
- Area of 1 mm^2 is calculated / counted
- Chamber is 0.02 mm deep

- Bacteria / mm^3 = number / square x 25 squares x 50
- Number of bacteria per cm^2 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