

# BACTERIAL GROWTH & PHYSIOLOGY

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# Growth

- It is an increase in all the cell components, which ends in multiplication of cell leading to an increase in population.
- It involves - an increase in the size of the cell & an increase in the number of individual cells.
- Bacteria divide by binary fission.



# Generation time

- Interval of time between two cell divisions
- OR
- The time required for a bacterium to give rise to 2 daughter cells under optimum conditions
- Also called population doubling time.



# Bacteria grow exponentially

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 ( $2^8$ )
0.5	2	4.5	512 ( $2^9$ )
1	4	5	1,024 ( $2^{10}$ )
1.5	8	5.5	2,048 ( $2^{11}$ )
2	16	6	4,096 ( $2^{12}$ )
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 ( $2^{19}$ )

Figure 6-6a Brock Biology of Microorganisms 11/e  
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Most bacteria divide in a short amount of time and produce a large amount of bacteria – easier to represent these large numbers by logarithmic scales



# Generation time

- Coliform bacilli like *E.coli* & other medically important bacteria – 20 mins
- Tubercle bacilli – 20 hrs
- Lepra bacilli – 20 days



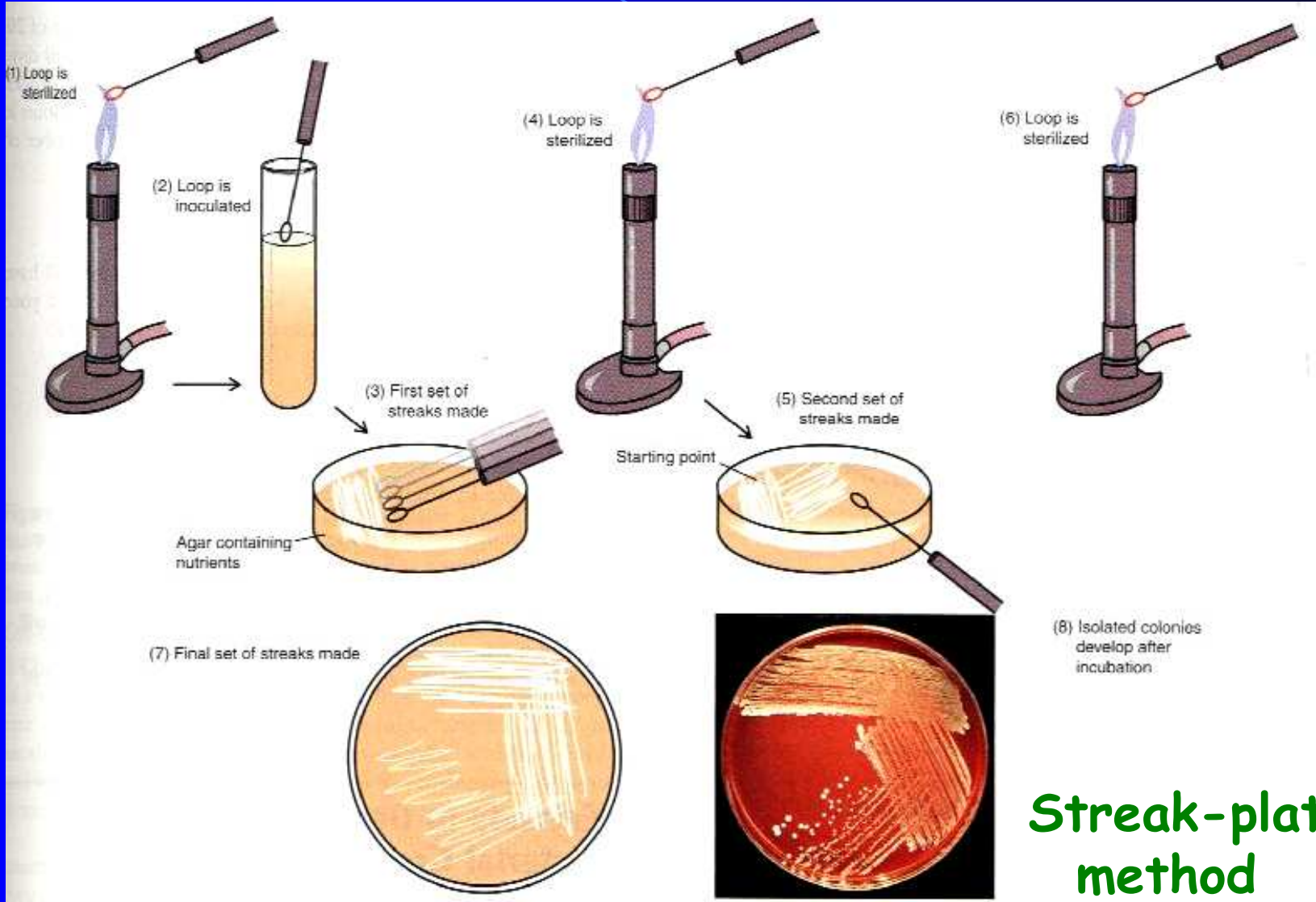
# Growth form in Laboratory

- Colony – formed by bacteria growing on solid media. (20-30 cell divisions)
- Each bacterial colony represents a clone of cells derived from a single parent cell.
- Turbidity – liquid media
- -  $10^7$ - $10^9$  cells/ml
- Biofilm formation – thin spread over an inert surface.





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# Streak-plate method





# Bacterial counts

- Growth in numbers can be studied by bacterial counts.
- 2 methods – Total cell count
- - Viable cell count



# Total Count

- Total number of cells in the sample – living + dead.
- Can be obtained by :
- Direct counting under microscope using counting chambers.
- Counting in an electronic device – Coulter counter



# Total Count

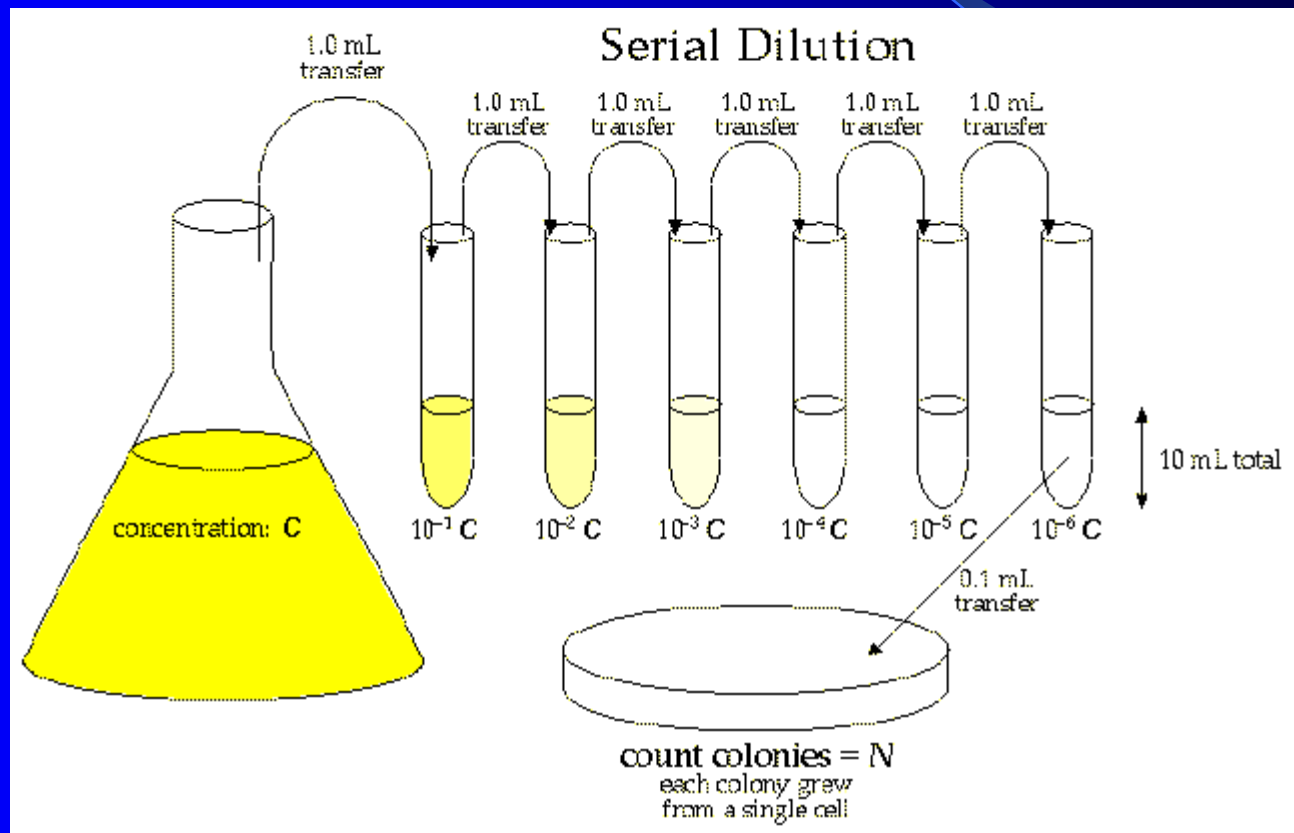
- Can be obtained by :
- Direct counting using stained smears - by spreading a known volume of culture over a measured area of slide.
- Opacity measurements using an absorptiometer/ nephelometer.
- Chemical assays of cell components.



# Viable Cell Count

- Measures the number of living cells.
- Methods – Surface colony count
- Dilution method
- Plating method
- Number of colonies that develop after incubation gives an estimate of the viable count.





# Bacterial Growth Curve

- When a bacterium is added to a suitable liquid medium & incubated, its growth follows a definite course.
- If bacteria counts are made at intervals after inoculation & plotted in relation to time, a growth curve is obtained.
- Shows 4 phases : Lag, Log or Exponential, Stationary & phase of Decline.



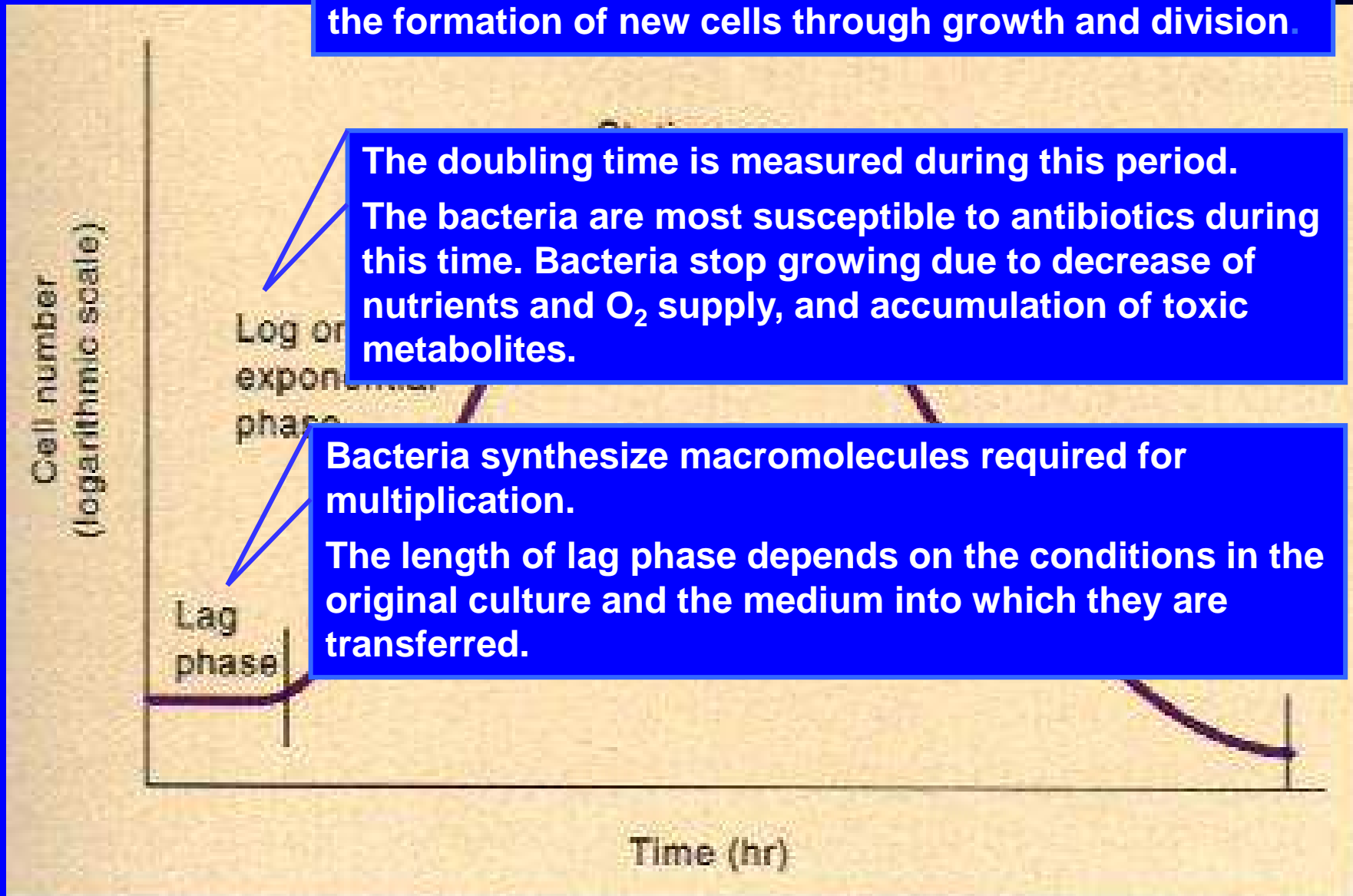
A balance between slow loss of cells through death and the formation of new cells through growth and division.

The doubling time is measured during this period.

The bacteria are most susceptible to antibiotics during this time. Bacteria stop growing due to decrease of nutrients and  $O_2$  supply, and accumulation of toxic metabolites.

Bacteria synthesize macromolecules required for multiplication.

The length of lag phase depends on the conditions in the original culture and the medium into which they are transferred.



# Phases of Growth Curve

- Lag phase – No increase in number but there may be an increase in the size of the cell.
- Log OR Exponential phase – cells start dividing and their number increases exponentially.



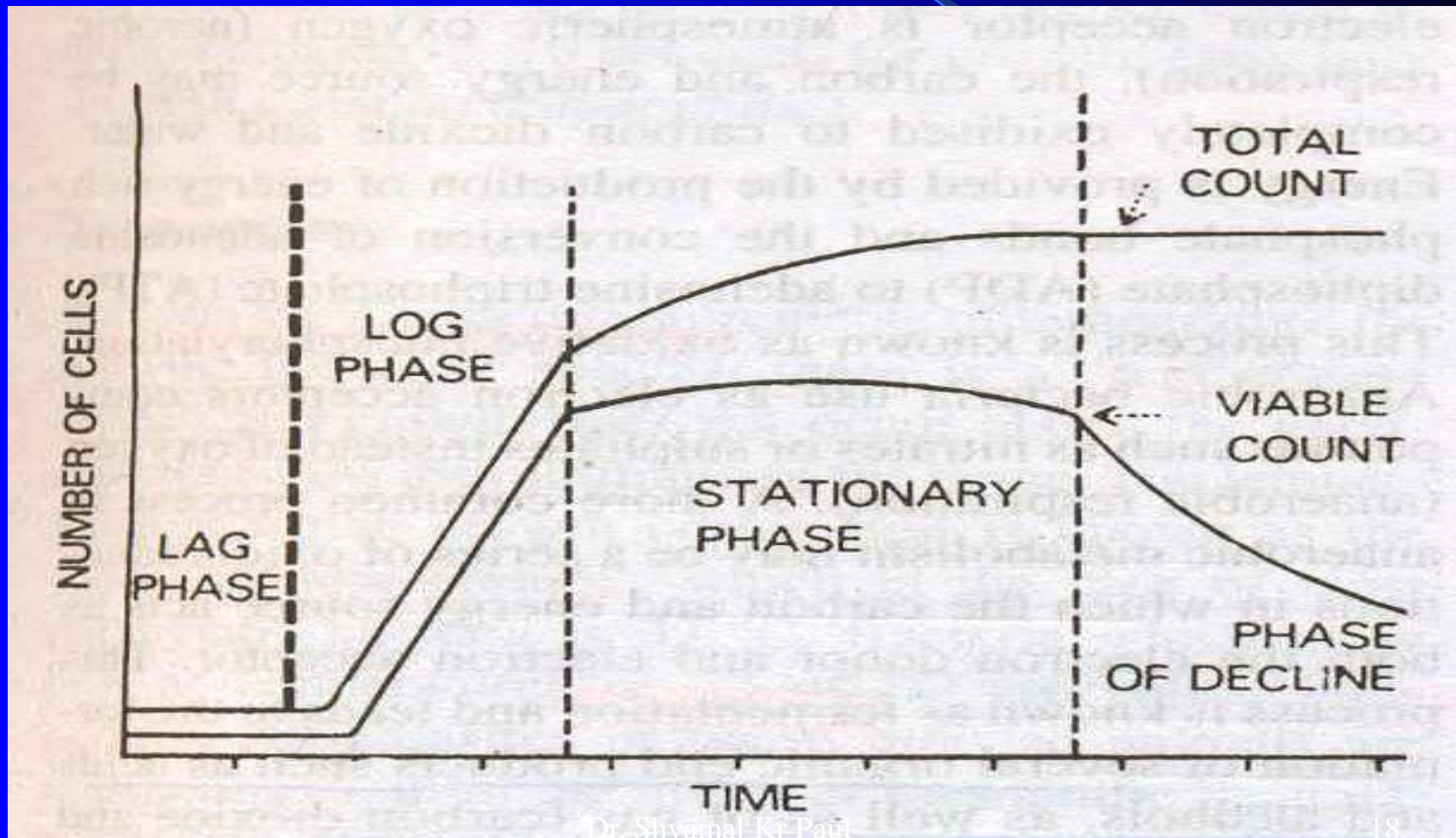


# Phases of Growth Curve

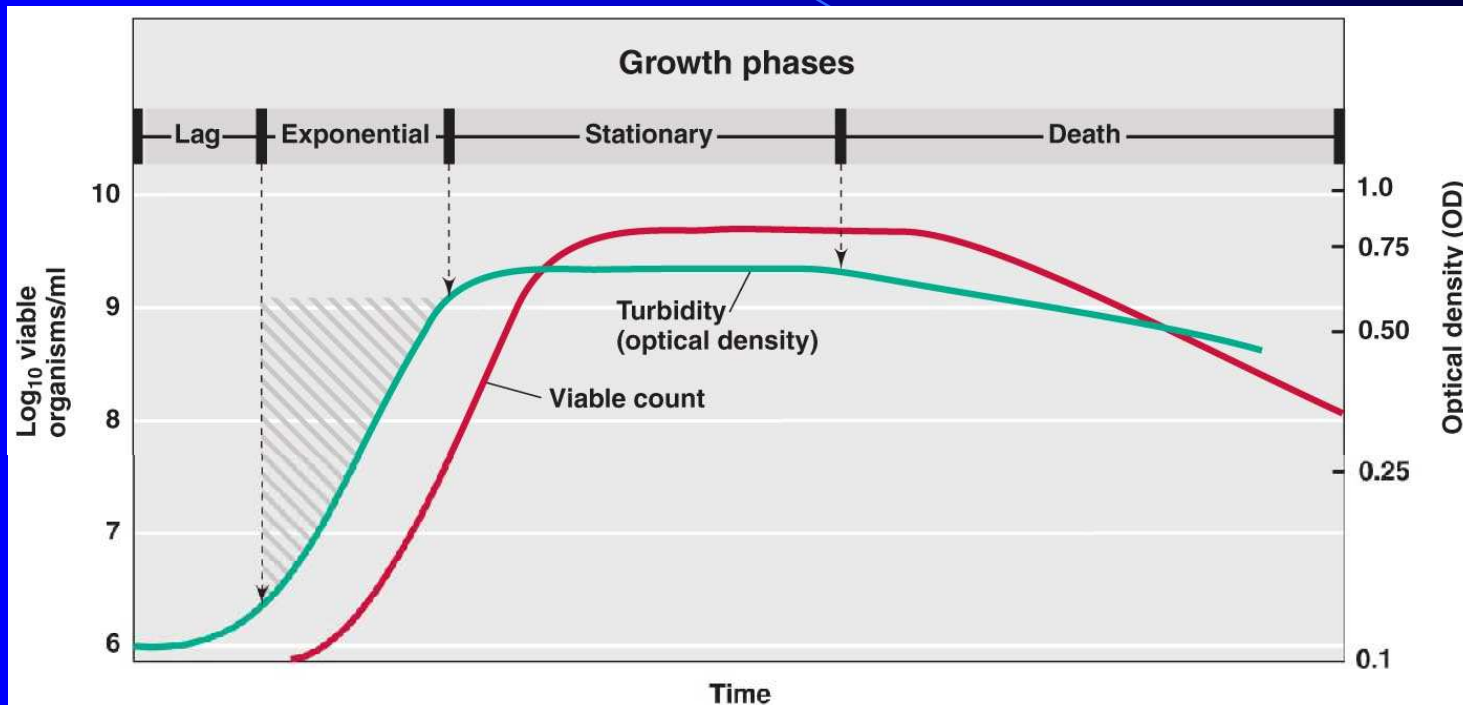
- Stationary phase – cell division stops due to depletion of nutrients & accumulation of toxic products.
- - equilibrium exists between dying cells and the newly formed cells, so viable count remains stationary
- Phase of Decline – population decreases due to the death of cells – autolytic enzymes.



# Phases of Growth Curve



# Growth Curve



Plot log cell concentration over time  
Plot OD versus time for comparison here



## Morphological & Physiological alterations during growth

- Lag phase – maximum cell size towards the end of lag phase.
- Log phase – smaller cells, stain uniformly
- Stationary phase – irregular staining, sporulation and production of exotoxins & antibiotics



# Factors Affecting Bacterial Growth

- Temperature
- Atmosphere – O<sub>2</sub> & CO<sub>2</sub>
- H-ion concentration
- Moisture & drying
- Osmotic effects
- Radiation
- Mechanical & sonic stress.



# Temperature

- Vary in their temperature requirements.
- Temperature range – growth does not occur above the maximum or below the minimum.
- Optimum Temperature – growth occurs best, 37°C for most pathogenic bacteria.

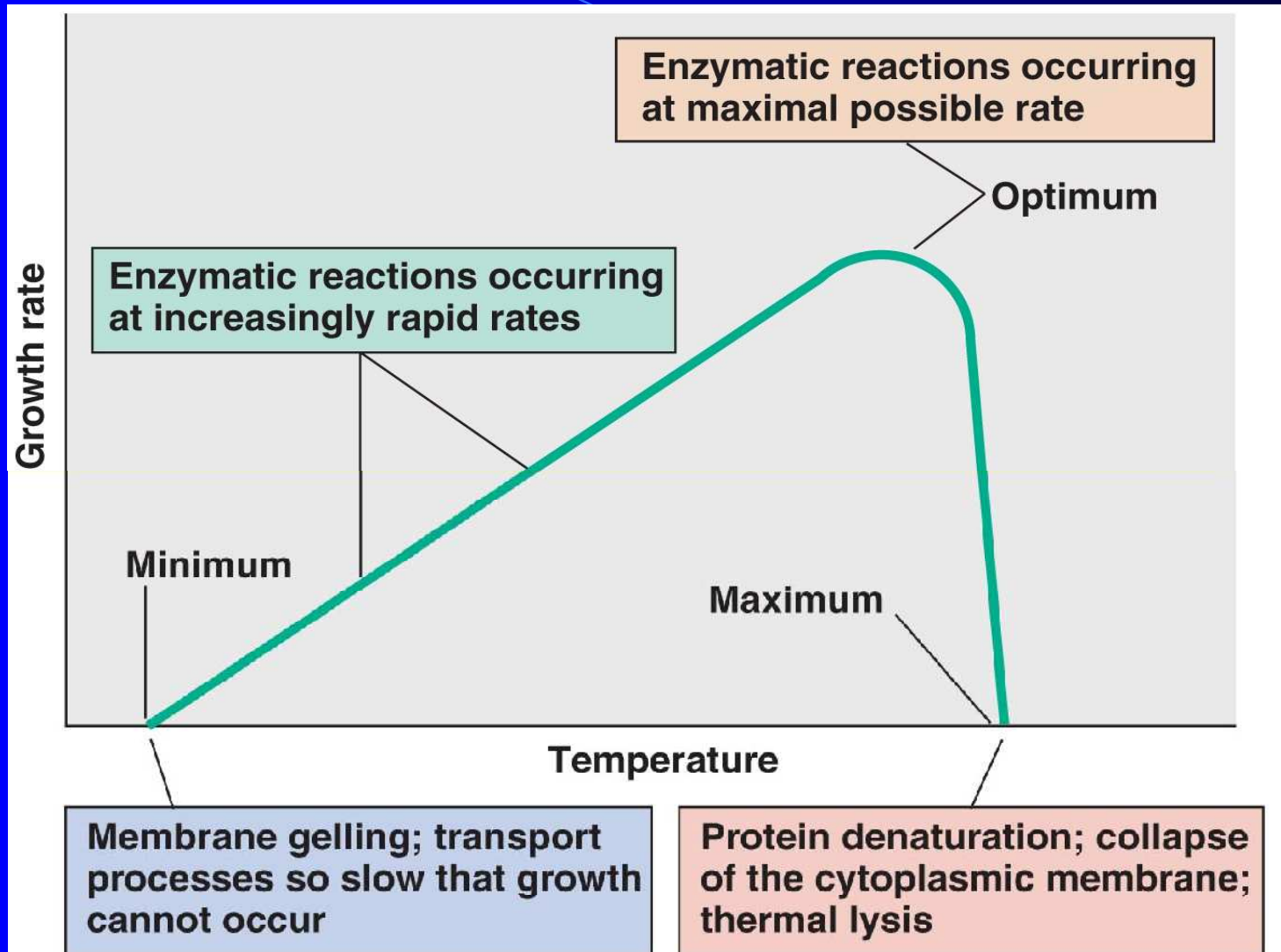


# Temperature

- Mesophilic – grows best between 25°C and 40°C.
- e.g. most bacterial pathogens
- Psychrophilic (cold loving) – grows best below 20°C
- e.g. *Flavobacterium* spp
- Thermophilic – grows best at high temp, 55- 58°C
- e.g. *Bacillus stereothermophilus*

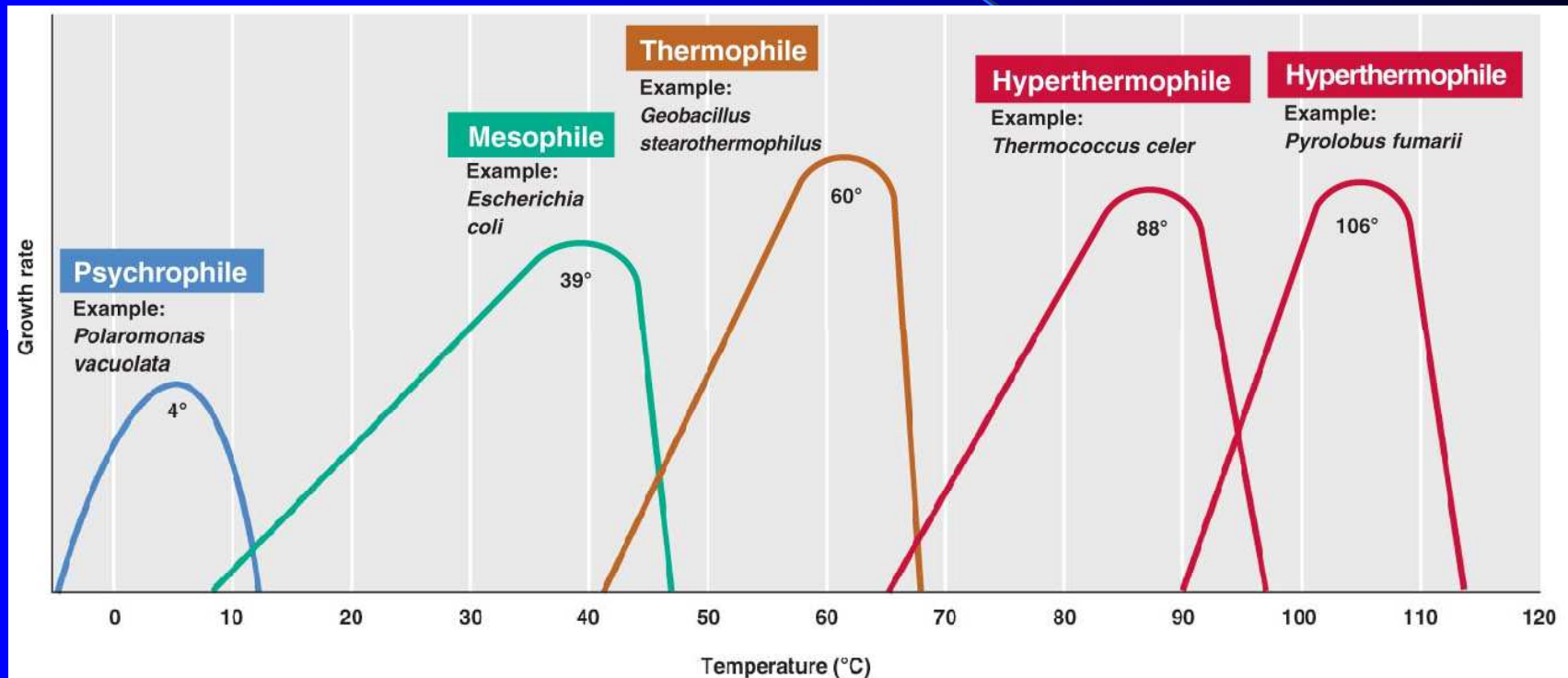


# The Cardinal Temperatures





# Temperature Requirements



# Atmosphere

- Depending on the O<sub>2</sub> requirement, bacteria are divided into :
- Strict (Obligate) Aerobes – require O<sub>2</sub> for growth e.g. *Pseudomonas aeruginosa*
- Strict (Obligate) Anaerobes – grow in the absence of O<sub>2</sub> & may even die on exposure to O<sub>2</sub> e.g. *Bacteroides fragilis*
- Microaerophilic – grow best in the presence of low oxygen levels
- e.g. *Campylobacter* spp, *Helicobacter* spp



# Atmosphere

- Facultative anaerobe – aerobic but can also grow in the absence of O<sub>2</sub>  
e.g. *Staphylococcus* spp
- Aerotolerant anaerobe – anaerobic, but tolerates exposure to O<sub>2</sub>  
e.g. *Clostridium perfringens*
- Capnophilic organism – requires high CO<sub>2</sub> levels e.g. *Meisseria* spp



## Toxic Forms of Oxygen

- Products of  $O_2$  metabolism  $\rightarrow$  toxic
  - Singlet oxygen:  $O_2$  boosted to a higher-energy state
  - Superoxide free radicals:  $O_2^-$
  - Peroxide anion:  $O_2^{2-}$
  - Hydroxyl radical ( $OH\bullet$ )

Reactants	Products
$O_2 + e^- \rightarrow O_2^-$	Superoxide
$O_2^- + e^- + 2 H^+ \rightarrow H_2O_2$	Hydrogen peroxide
$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH\bullet$	Hydroxyl radical
$OH\bullet + e^- + H^+ \rightarrow H_2O$	Water

Outcome:

$$O_2 + 4 e^- + 4 H^+ \rightarrow 2 H_2O$$

Figure 6.29

# Toxic Forms of Oxygen

- Organisms that use aerobic metabolism must detoxify these products
  - Catalase enzyme:  $2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$
  - Peroxidase enzyme:  $\text{H}_2\text{O}_2 \rightarrow 2 \text{H}^+ + \text{H}_2\text{O}$
  - Superoxide dismutase enzyme: detoxifies  $\text{O}_2^-$  and  $\text{OH}^\bullet$
  - Obligate anaerobes lack these enzymes

# H-ion Concentration

- pH range, optimum pH
- Neutral or slightly alkaline pH (7.2 – 7.6) – majority of pathogenic bacteria grow best.
- Lactobacilli – acidic pH
- *Vibrio cholerae* – alkaline pH



# Moisture & Drying

- Water – essential ingredient of bacterial protoplasm. Hence drying is lethal to cells.
- Effect of drying varies :
  - *T.pallidum* – highly sensitive
  - *Staphylococci* sp– stand for months
- Spores – resistant to dessication, may survive for several decades.



# Osmotic effects

- More tolerant to osmotic variation due to mechanical strength of their cell walls.
- Radiation
- X rays & gamma rays exposure – lethal
- Mechanical & Sonic Stress
- May be ruptured by mechanical stress.





# Bacterial Nutrition

- Water constitutes 80% of the total weight of bacterial cells.
- Proteins, polysaccharides, lipids, nucleic acids, mucopeptides & low molecular weight compounds make up the remaining 20%.
- For growth & multiplication, the minimum nutritional requirements are water, a source of carbon, a source of nitrogen & some inorganic salts.



## Classification of Bacteria Based on Nutritional Requirement

- Phototrophs – Bacteria which derive their energy from sunlight.
- Chemotrophs – Bacteria which derive energy from chemical reactions.
- Organotrophs : require organic sources of hydrogen
- Lithotrophs : require inorganic sources of hydrogen like  $\text{NH}_3$ ,  $\text{H}_2\text{S}$



## Classification of Bacteria Based on Nutritional Requirement

- Based on the utilization of carbon compounds, bacteria are classified as :
  1. **Autotrophs** - can synthesise all their organic compounds by utilising atmospheric  $\text{CO}_2$  &  $\text{N}_2$ . No medical importance.
  2. **Heterotrophs** - unable to synthesise their own metabolites & depend on preformed organic compounds.



# Growth Factors

- Some bacteria require certain organic compounds in minute quantities – Growth Factors OR Bacterial Vitamins.
- It can be :
- Essential – when growth does not occur in their absence.
- Accessory – when they enhance growth, without being absolutely necessary for it.



# Growth Factors

- Identical with mammalian nutrition
- Vitamin B complex –
- thiamine
- riboflavine
- nicotinic acid
- pyridoxine
- folic acid &
- Vit.B 12

