GROWTH REQUIREMENT OF BACTERIA

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Growth requirements

- To identify & study the bacterial species it is necessary to grow an organism under laboratory conditions
- Two conditions must be fulfilled
  - Suitable nutrient supply (chemical req.)
  - Physical condition

OBJECTIVES

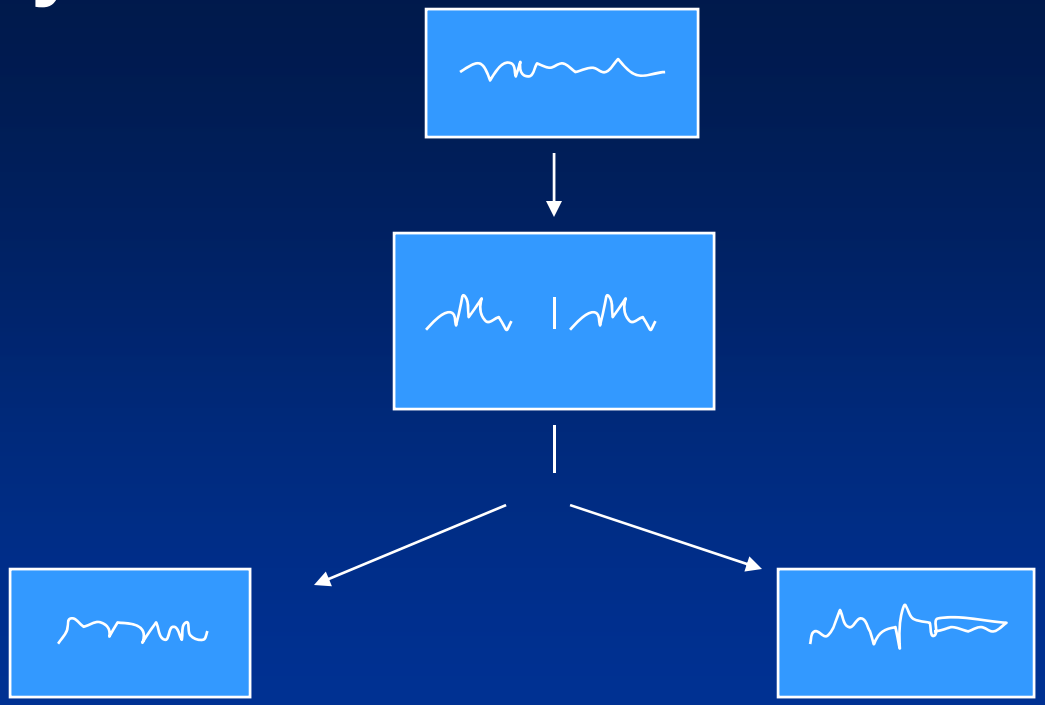
- Growth
- Batch culture
- Continuous culture
- Microbial physiology
- Microbial metabolism
- Oxygen requirement
- Nutrients for growth
- Effect of environmental factors

Growth

- When bacterial cell are kept into a suitable nutrient medium & incubated under appropriate conditions, almost all of the bacterial cells have potential to grow at very rapid rate.

- What is growth?
  - Increase in all the components of an organism
  - Increase in size of bacterium
  - Increase in number

- **Binary fission**



generation time

- **The time required for a bacterium to give rise to two daughter cells under optimum conditions.**

Example- In Coli form bacilli generation time is 20 min

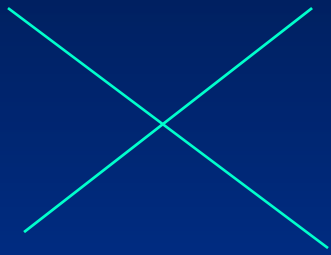
From 1 bacillus

↓

After 20 min. 2 cells.

↓

After 24 hrs. 1x 10^{21} cells.



This is not true. Why ?

- Because of insufficiency of nutrients or special growth factors
- Effects of toxic products

-In host- various host defense mechanisms

Batch culture

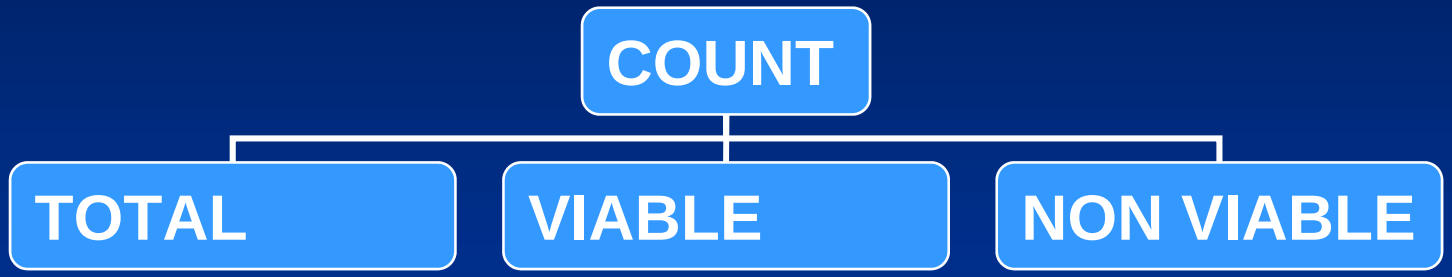
- **Usual method of growing bacteria in laboratory**

- **When bacteria are inoculated in a vessel containing liquid medium having req. nutrients, under optimum cond. is kn. as Batch culture**

Continuous culture

- Open system in which there is continuous supply of fresh nutrients into the culture vessel and a continuous removal of grown bacteria by means of a constant -level device (chemostat)
- Pathogenic bacteria- intermediate situation
- Bacteria growing on solid media-colonies
- Liquid media- diffuse

Bacterial cell count



Methods to count Total Count

- Direct counting under the microscope using counting chambers
- Counting in an electronic device as in the Coulter counter
- Direct counting using stained smears prepared by spreading a known volume of the culture over a measured area of a slide

Contd.

- Comparing relative numbers in smears of the culture mixed with known number of other cells
- By opacity measurement using absorptiometer or nephlometer
- By separating the cells by centrifugation or filtration and measuring their wet or dry wt.
- Chemical assay of cell components such as nitrogen

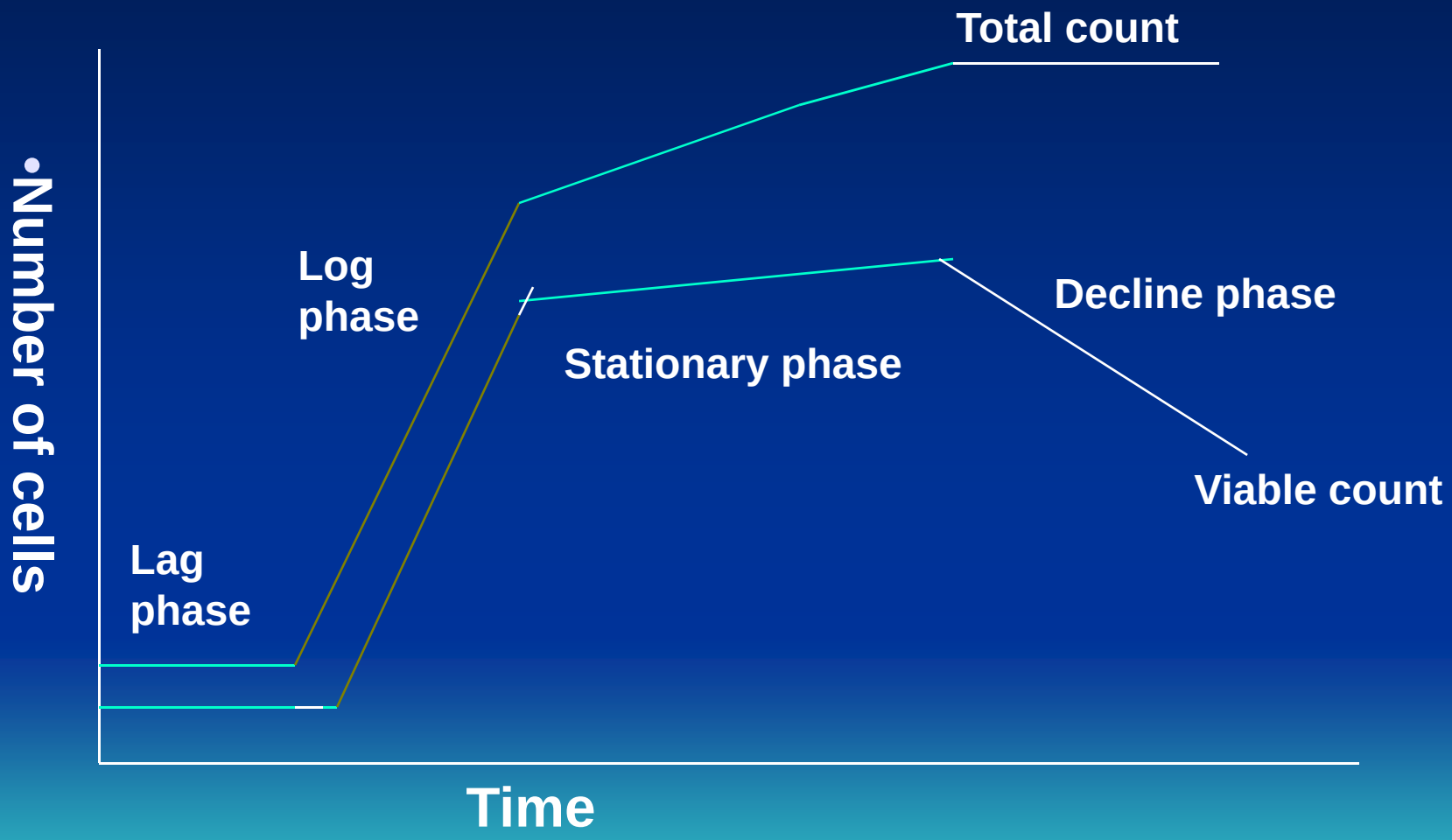
Methods to count Viable Count

- **Dilution method**
- **Plating method**
  - **Streaking method**
  - **Pouring method**

❖ **Colony forming units**

- **If number of bacterial cells present at different times after inoculation is measured and the number is plotted in relation to the period of growth, the resultant plot is referred as a (bacterial) batch growth curve.**

Bacterial (batch) growth curve



Lag phase

- Increase in size of cells, Not in number
- Period required to adapt a new environment & to built up necessary enzymes and metabolites
- Duration of period varies with species, size of inoculation, nature of culture medium & environmental factors like temp.

Log phase

- Continuous cell growth
- Cell divides continuously
- Straight line on plot
- Depend on generation time of the bacterium
- Cells are smaller and stain uniformly
- Not true in vivo

Stationary phase

- Decrease rate of multiplication
- No growth no death
- Balance between reproduction & death
- Why ?
  - Exhaustion of an essential nutrients in the medium
  - Accumulation of toxic waste products (e.g. organic acids - ↓ $p^{H)}$

Contd.

- **Cells**

  - exhibit a corresponding variation in morphology & physiology

  - have high level of I/C storage polymers such as polysaccharides, lipid
- **Many species produce secondary metabolites such as antibiotics, exotoxins**
- **Some spore forming bacteria starts sporogenesis**

Decline or Death phase

- Cell population decreases due to cell death
- Divergence between total count & viable count on plot
- Why ?
  - Accumulation of toxic waste products
  - Autolysis

❖ Rapidity of the onset of the death phase is an imp. factor that may influence the spread of infection

Microbial study

Chemical composition

Principle constitute of bacterial cell is water.

Water represents 80% of the total wt.

Proteins, Polysaccharides, lipids,

Peptidoglycan, Nucleic acid, Low mol. Wt. components make up the rest.

Bacterial meta. Is similar to the meta. of higher organisms.

Unity of biochemistry.

- **Energy source**
  - **Light** → **PHOTOTROPHS**
  - chemical (**Oxidation of inorganic/organic**) → **CHEMOTROPHS**
- **Hydrogen/Electron source**
  - → **LITHOTROPHS**
  - → **ORGANOTROPHS**

Nutritional classification

- **Depends on Carbon, Energy, Hydrogen/Electron source**

❖ **C source**

→ CO_2 → **AUTOTROPHS**

→ **Reduced, preformed, organic molecules from other organisms**

↓

HETEROTROPHS

↓

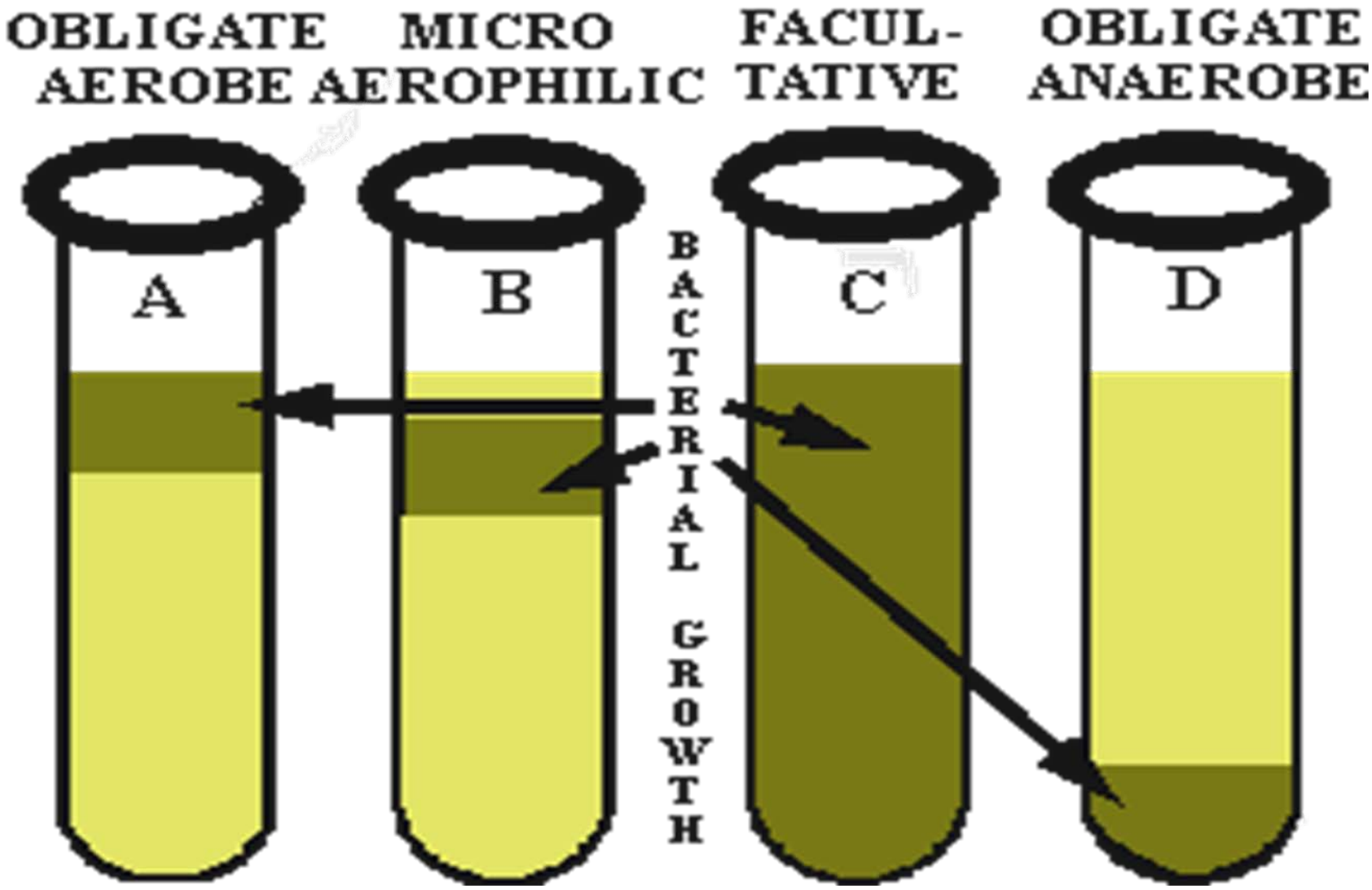
Medically imp. bacteria

Nutrients required

- Water
- Macro elements – C, O, N, S, H, P
- Microelements – K, Ca, Mg, Iron
- Trace amt. of manganese, cobalt, copper etc.
- Growth factors- essential, accessory

Oxygen requirement & Metabolism

- **Aerobic bacteria - require oxygen for their growth**
- **Obligate aerobe - grow only in presence of oxygen**
- **Facultative anaerobe - are ordinarily aerobic but can grow anaerobically, though less abundantly**
- **Anaerobic bacteria - grow in absence of oxygen**


OBLIGATE AEROBE
MICRO AEROPHILIC
FACUL-TATIVE
OBLIGATE ANAEROBE
A
B
C
D
BACTERIAL GROWTH

Contd.

- **Obligate anaerobe - grow only in absence of oxygen, may even killed in its presence**
- **Microaerophilic – grow best in the presence of a low oxygen tension.**
- **Aerobic bacteria uses oxygen as a hydrogen acceptor (oxidative phosphorylation)**

Microbial metabolism

- **Conversion of the CARBON nutrients into basic ‘ building blocks ‘ to be used in biosynthesis**

- **Conversion of ADP ⟶ ATP**

Physical conditions

1. **Temperature**

   - Most of the microorganisms grow well at the temp. favored by humans

- **Psychrophiles (cold-loving microbes) bacteria that grows best below 20^0 C**
- **Mesophiles (moderate-temp.-loving) bacteria which grow best at temp. of 25-40^0 C**
- **Thermophiles (heat-loving) bacteria grow best at high temp., 55-80^0 C**

2.pH

*** pH refers to the acidity or alkalinity of a solution.**

*** most of the pathogenic bacteria grow at neutral or slightly alkaline pH. (except V. cholerae & Lactobacilli)**

*** optimum pH – pH at which bacteria grow best.**

3. Light

*** Bacteria grow well in dark except phototropic spp.**

*** Sensitive to UV light, sunlight, radiations**

4. Osmotic effect

Plasmolysis – exposure to hypertonic solution leads to osmotic withdrawal of water & shrinkage of protoplasm.

Plasmoptysis – sudden transfer from concentrated solution to distilled water leads to cell swelling & rupture.

5. Moisture & drying – T. pallidium & staphylococci


THANK YOU