Practical 4

Growth requirements

of

bacteria

Factors require for growth of bacteria

- Nutrients
- Oxygen
- Carbon Dioxide
- Temperature
- pH
- Moisture
- Light
- Osmotic effect

1. NUTRIENTS:

Carbon sources

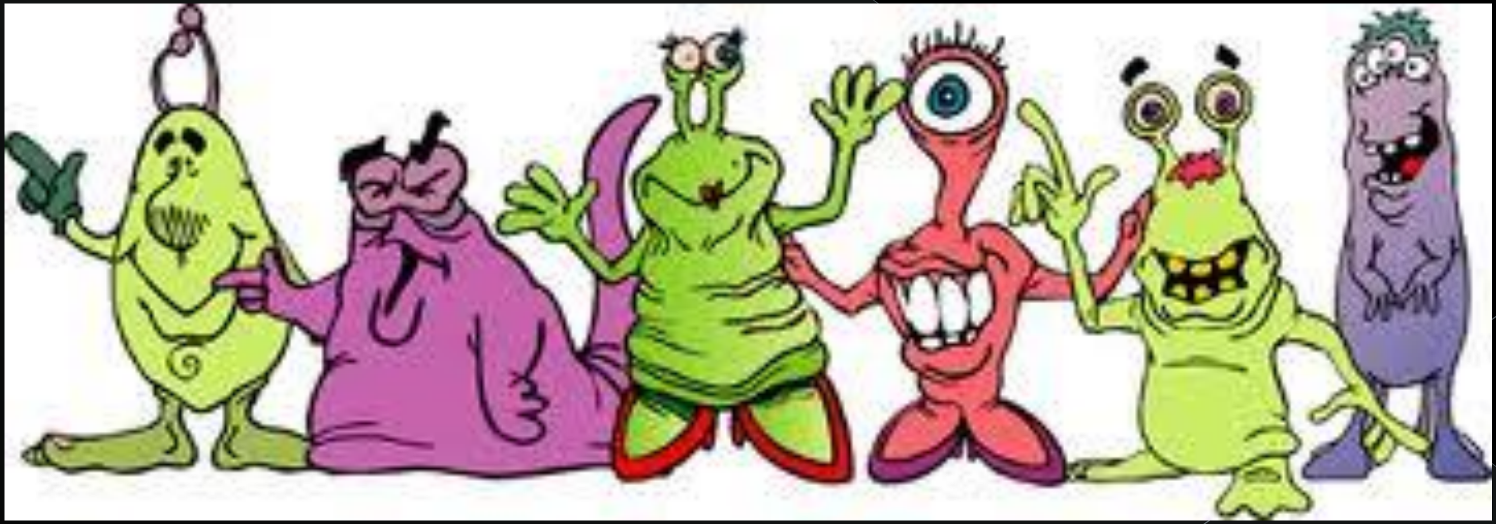
CO_2 = autotroph

organic = heterotroph

Energy sources

sunlight = phototroph

organic = chemotroph



“Chemoheterotroph

Derive both carbon and energy from organic compounds

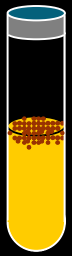
Chemo autotroph

Derives energy from organic compounds and carbon source from inorganic compounds

Oxygen

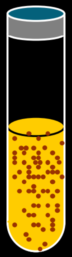
Obligate aerobes

- Only aerobic growth, oxygen required



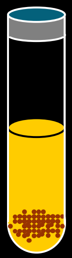
Facultative anaerobes (most human pathogens)

- Greater growth in presence of oxygen



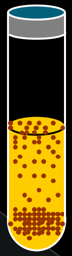
Obligate anaerobes

- Only anaerobic growth, cease with oxygen



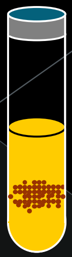
Aerotolerant anaerobes (e.g., *C. perfringens*)

- Only anaerobic growth, continues with oxygen



Microaerophiles (e.g., *M. tuberculosis*)

- Only aerobic growth with little oxygen

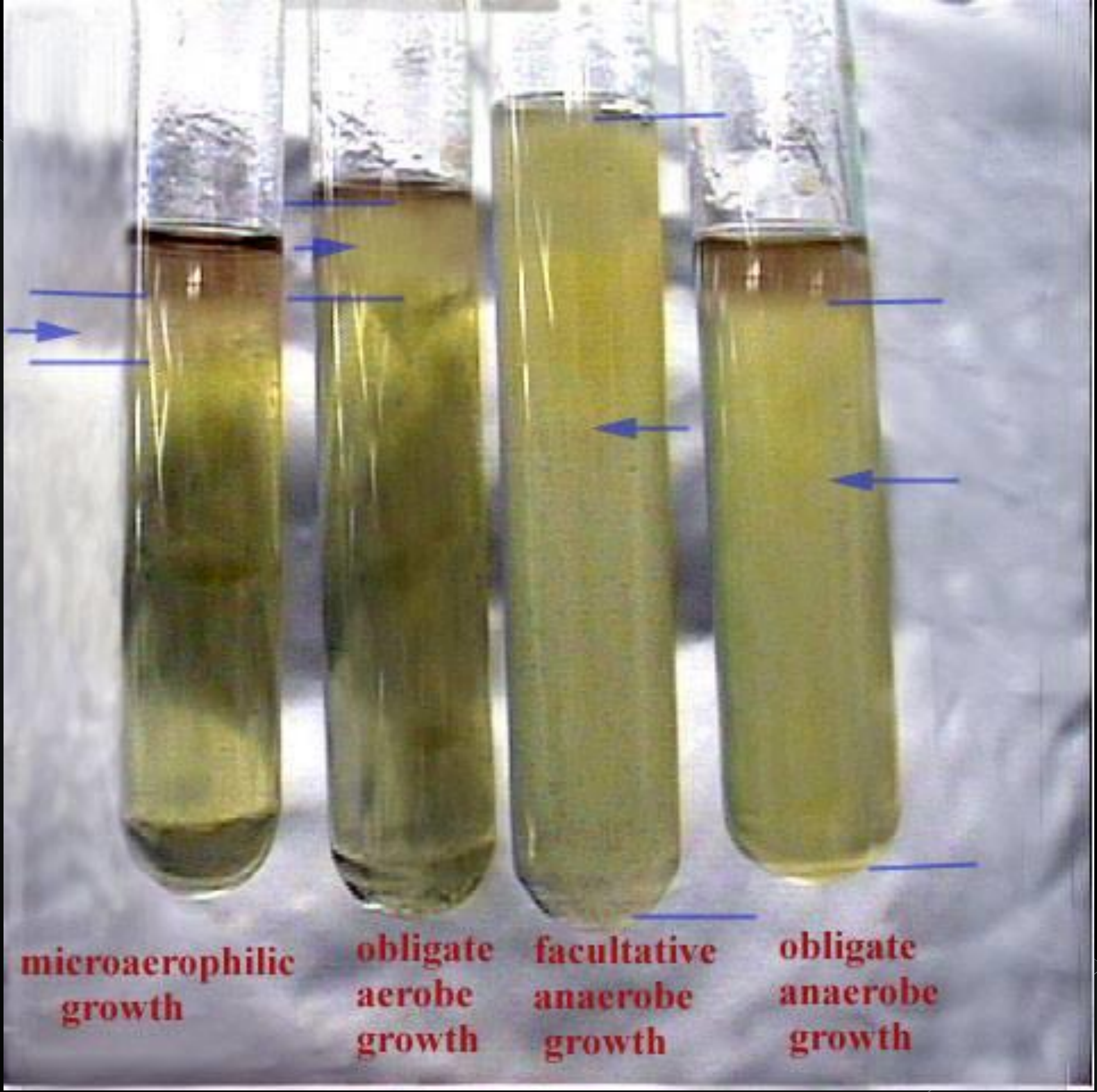


2. OXYGEN

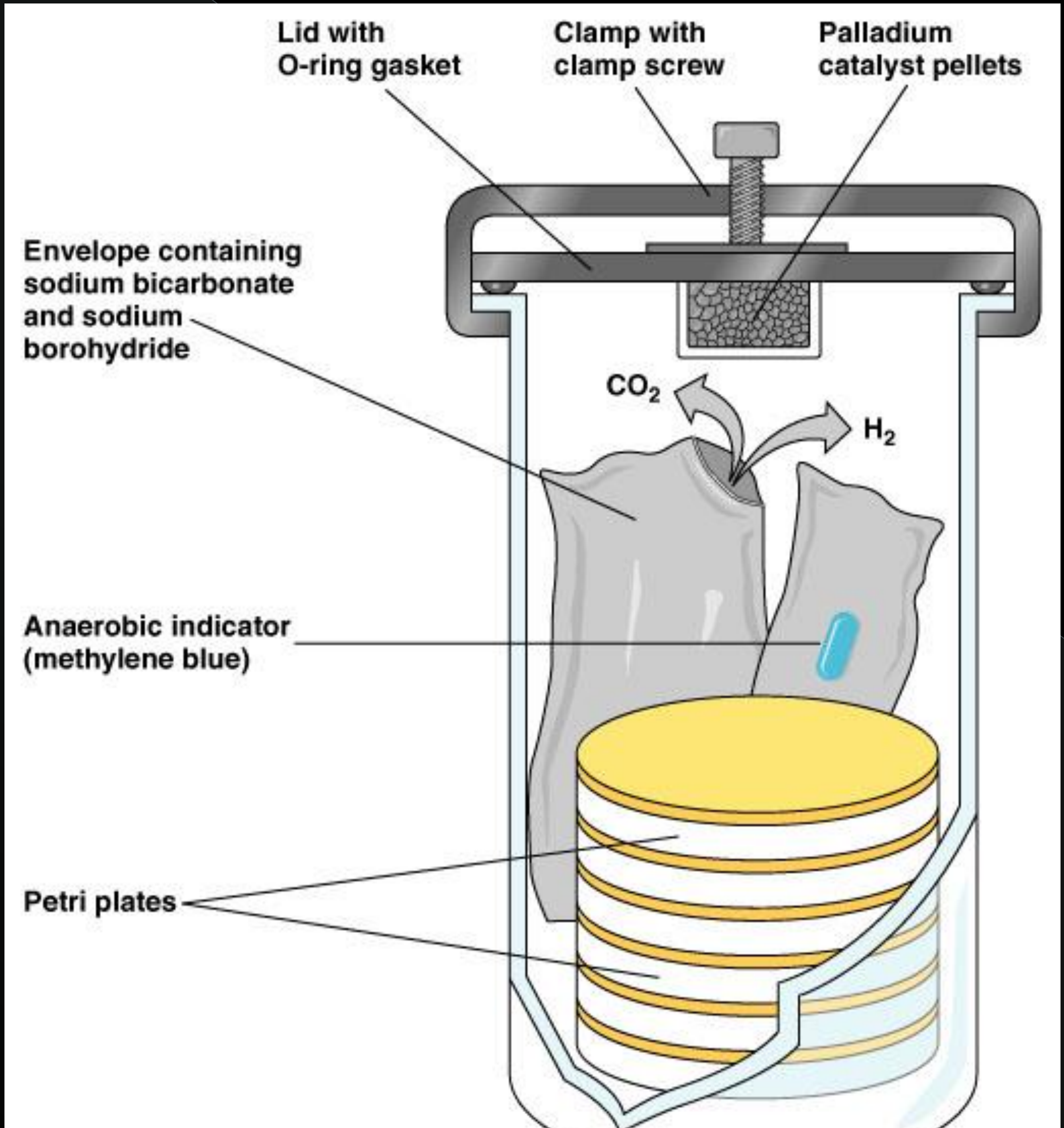
- **Aerobes:**grow well in the presence of normal atmospheric oxygen . e.g. most fungi, protozoa and bacteria such as genus Bacillus,Vibrio,Pseudomonas.
- **Microaerophile**: Require small amount of oxygen e.g. Campylobacter sp & Helicobacter sp.
- **Facultative anaerobes**: Ordinarily aerobe, grow in absence/presence of oxygen e.g. enteric bacilli and Staphylococci
- **Aerotolerant anaerobes**: Do not utilize oxygen but can survive in its presence. e.g. Lactobacilli and anaerobic Streptococci.
- **Obligate anaerobes**: Cannot grow in normal atmospheric oxygen. e.g. Clostridium tetani., Bacteroides spp.

2. OXYGEN & 3. CARBON DIOXIDE:

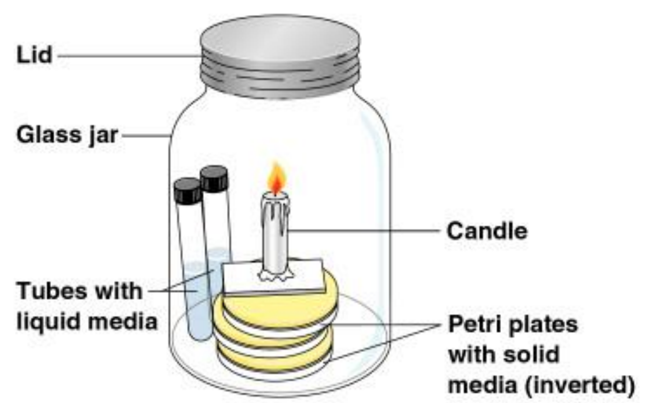
		Environment	
Group	**Aerobic**	**Anaerobic**	**O_2 Effect**
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth	Toxic
Facultative (An)aerobe	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized


microaerophilic growth
obligate aerobe growth
facultative anaerobe growth
obligate anaerobe growth

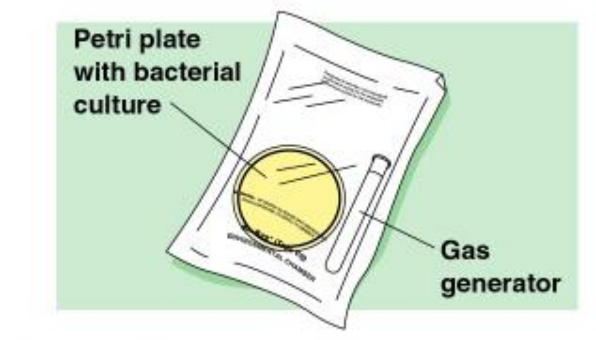
Anaerobic and Low O_2 Culture Methods



Brewer or anaerobic jar



Candle jar



CO_2 packet

Cultivation in vaccum:

- *By cultivating the culture media in vaccum desiccator.*

  Dis adv: Some O_2 always left.

  Media get detached from the plates.

Displacement of Oxygen

- *Anaerobiosis can be achieved by displacement of oxygen with other gases like hydrogen, nitrogen, helium, carbon dioxide.*
- ***Candle jar:***

Provides 5-10% CO_2

- *Capnophilic bacteria, e.g. Brucella abortus, Neisseriae species*
- *Not complete anaerobiosis achieved.*

Chemical method

Pyrogallic acid + sodium hydroxide in test tube placed inside an air tight jar with inoculated culture plates.

Pyrogallic acid will absorb the oxygen from the environment.

Dis adv.: Carbon monoxide which produce during mixture of reagent will inhibit the growth of organisms.

Instead of above reagent other chemical can be used like chromium and sulfuric acid mixture (Rosenthal method) Or yellow phosphorus.

Biological method

Absorption of oxygen from closed system can be achieved by incubating the culture with germinating seeds or chopped vegetables.

Dis adv: anaerbiosis produce by this method is slow and ineffective.

McIntosh-Fielde's anaerobic jar

- It is a glass or metal jar with metal lid which can be closed air tight with screw.
- Lid has two tubes with taps.
- One acts as gas inlet and other as a outlet.
- Lid has also two terminals which connected with electricity.
- At under surface of lid small porcelain spool wrapped with palladinised asbestos is suspended.
- The out let tube is connected with vaccum pump air inside is evacuated.
- The outlet tube is then closed and inlet tube connected to a hydrogen supply.
- The electric terminal connected with current supply so that palladinised asbestos is heated this will acts as a catalyst for hydrogen and oxygen.
- It ensure the complete anaerobiosis.
- But it carries the risk of explosion which can be eleminated by using alumina pellets coated with palladium in guaze sachet.

Gaspak

- *Gaspak is available as a disposable envelop containing chemical which generate hydrogen and carbon dioxide on addition of water.*
- *Inoculated plates are kept in jar, the gaspak envelop with water added kept inside and lid screwed tight.*
- *H_2 and CO_2 are liberated and with presence of cold catalyst H_2 combine with O_2 and forms the H_2O.*
- *It remains colourless anaerobically.*

Reduction of oxygen in medium

By following method

Using 1% glucose

0.1% Thioglycollate

0.1% Ascorbic acid

0.05% Cystein

Broth containing fresh animal tissue such rabbit kidney, spleen, testes, or heart (Smith Noguchi medium)

Robertson's cooked meat medium

Cooked meat in broth with a layer of sterile vaseline over it.
Contains 15 ml broth in 30 ml glass screw capped bottle,
Meat particles up to 2.5 cm height,
Unsaturated fatty acids take up oxygen which is catalyse by hematin in meat particals.
Sulphydril group which reduces O-R potential.
Glutathione and cystein utilize the O_2 in media.
It permits the growth of strict anaerobes and indicates their saccharolytic and proteolytic activities by meat being turned red and black respectively.
Blood agar containing vitamin K and Hemin used to culture anaerobes.
Reducing agar concentration will enhance the anaerobic growth by reducing O_2 diffusion in medium.

Robertson's cooked meat medium

Saccharolytic

Proteolytic

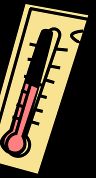
Anaerobic chamber (Glove box)

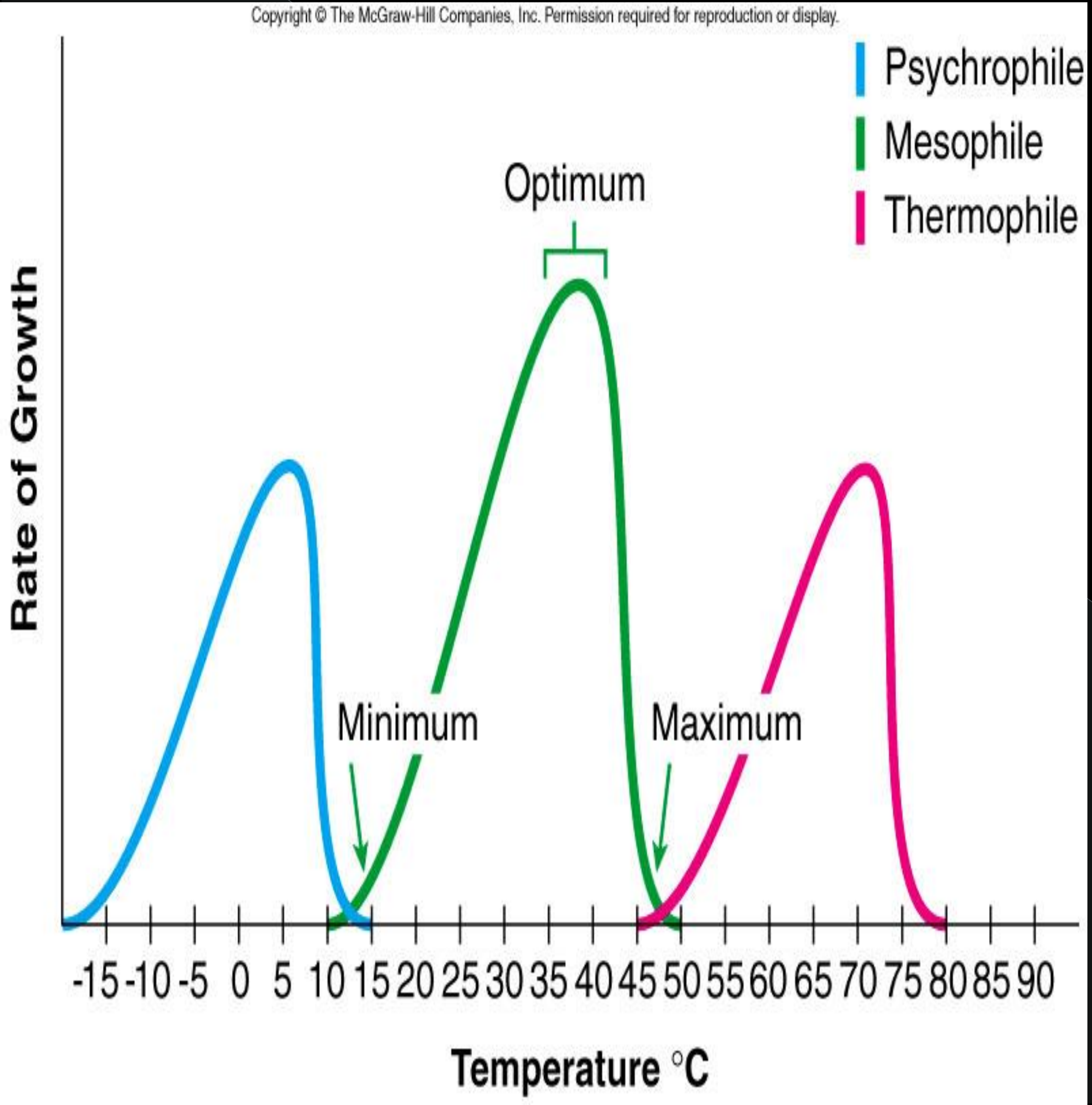
- *For the fastidious anaerobes pre reduced media and glove box or anaerobic chamber may be used.*
- *Anaerobic chamber is air tight cabinet,*
- *Filled with inert gas,*
- *Glove for the hands.*

4.TEMPERATURE

- Pathogenic bacteria grow best at body temperature, i.e. 37°C.
- **Psychrophiles**: Microorganisms that grow below 15°C and are capable of growing at 0°C. Room temperature is lethal to the organism.Ex. Saprophytes.
- **Mesophiles**: Microorganisms that grow at 20-40°C. Most human pathogens are mesophiles. Infects all warm blooded animals.largest group of bacteria.
- **Thermophiles**: Microorganisms that grow above 45°C. e.g. spore forming Bacillus stereothermophilus.

4. Temperature:





Psychrophiles :
0°c – 15°c

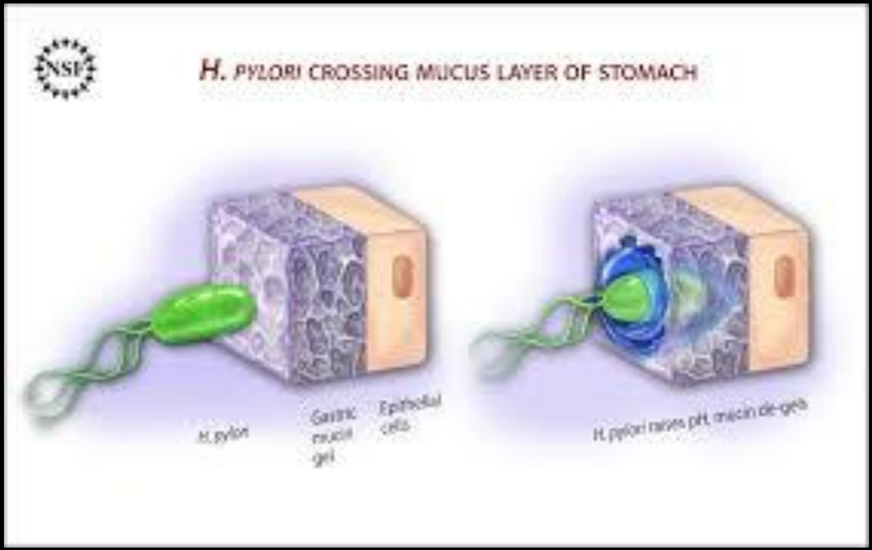
Mesophiles:
20°c - 40°c

Thermophiles:
> 45°c

5. pH

Acidophillic

- *Helicobacter pylori* lives in stomach under mucus layer
- Many bacteria and viruses survive low pH of stomach to infect intestines
- Lactobacilli



Basophillic

- Many bacteria grow best in alkali medium e.g. Vibrio cholerae

6. MOISTURE:

- Moisture is necessary for growth of bacteria.
- Some bacteria die quickly in dry conditions e.g.Gonococcus and Treponema pallidum.
- Some bacteria can survive in dry conditions e.g. Staphylococcus aureus and Tubercle bacilli
- Freeze drying & lyophilization.

7.LIGHT:

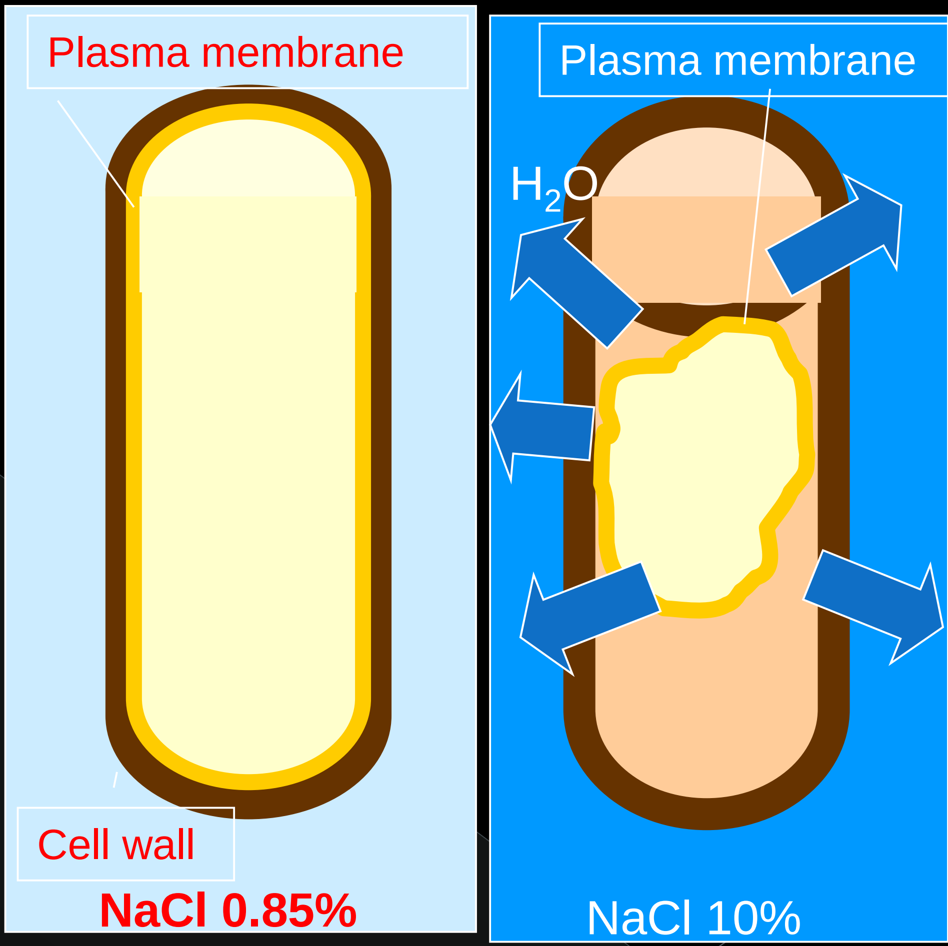
- **Bacterial growth and viability are favoured by darkness. Ultraviolet rays and Ionizing radiations quickly kill the bacteria.**
- Photo synthetic bacteria require light and photochromogenic mycobacteria produce pigment only when exposed to light.

8. Osmotic Effect:

Due to mechanical strength of cell wall, bacteria are able to tolerate osmotic variation but sudden exposure to hypertonic solution may cause plasmolysis and sudden transfer to distilled water may cause plasmoptysis.

Osmotic pressure

- High osmotic pressure (hypertonic) removes water causing plasmolysis – inhibits growth i.e. salt as preservative
- Low osmotic pressures (hypotonic) cause water to enter and can cause lysis
- Bacteria are more tolerant to osmotic variations because of the mechanical strength of the cell wall



Food

Acidity

Time

Temperature

Oxygen

Moisture

QUESTIONS:

Q-1 What is generation time? Give the generation time for E.coli, Mycobacterium tuberculosis & Mycobacterium leprae

ANS: The interval of time between two cell divisions, or the time required for a bacterium to give rise to two daughter cells under optimum conditions, is known as the generation time or population doubling ime.

a) E.coli: 20 minutes

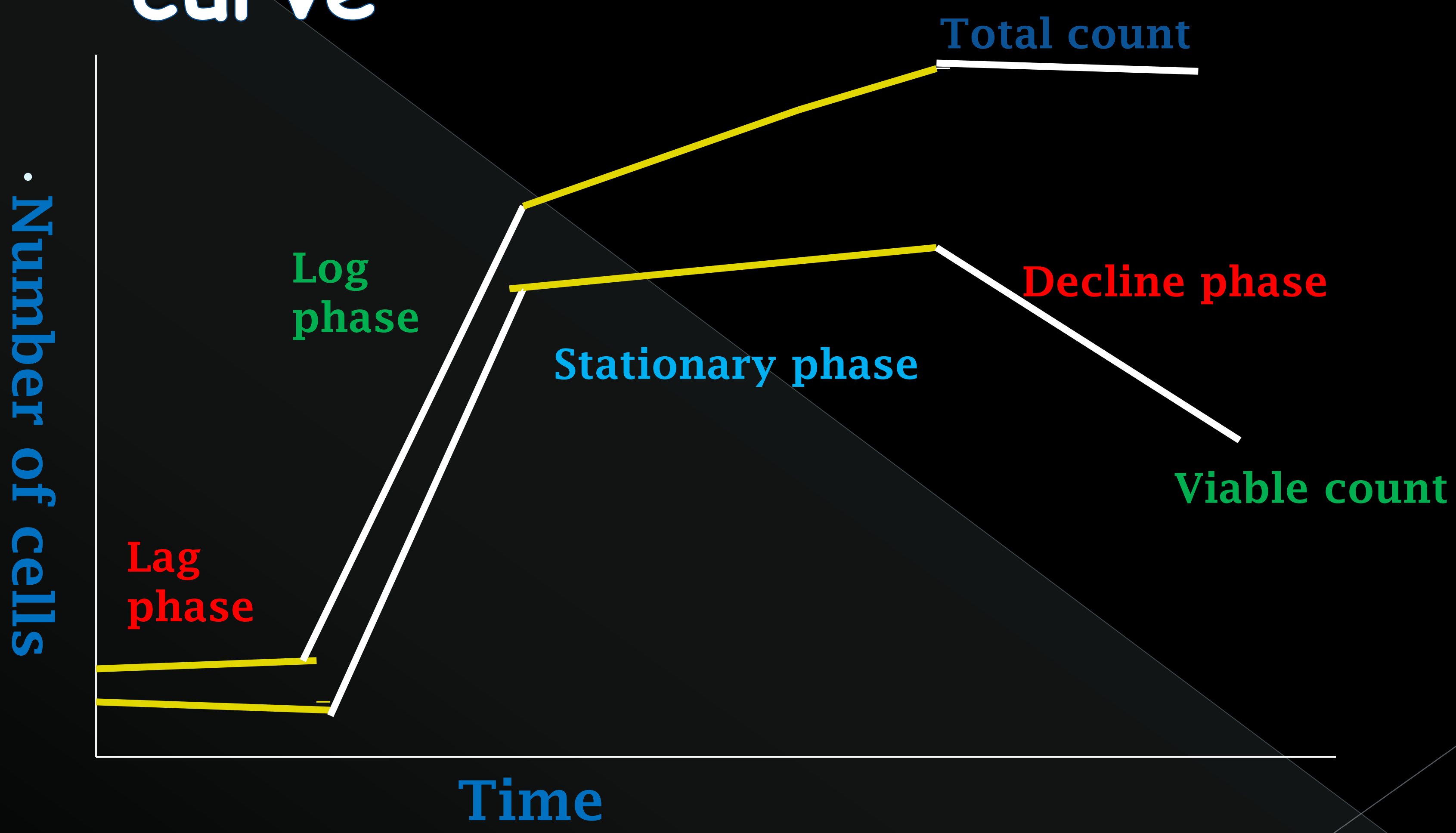
b) Mycobacterium tuberculosis: 20 hours

c) Mycobacterium leprae : 20 days.

Q-2: What is continuous culture?

Ans : 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)

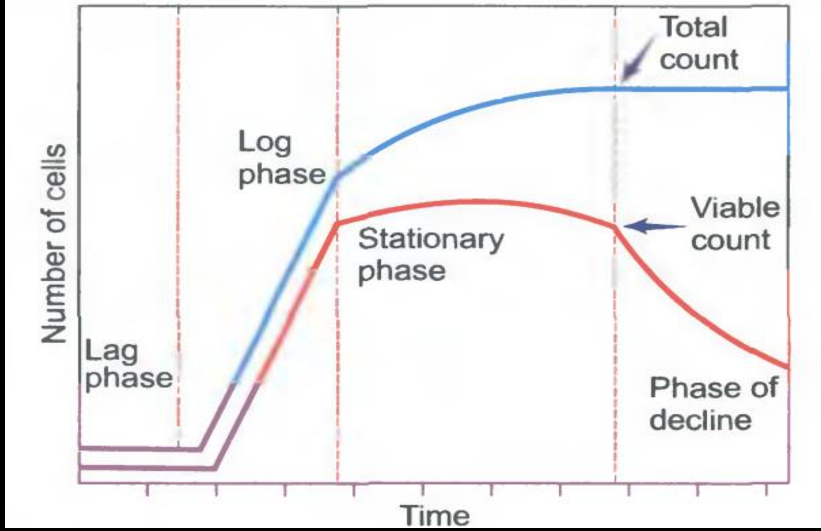
Bacterial (batch) growth curve



Bacterial Growth curve

- When a bacterium is inoculated into a suitable liquid culture medium and incubated, its growth follows a definite course.
- When bacterial count of such culture is determined at different intervals and plotted in relation to time, a ***bacterial growth curve*** is obtained comprising of four phases.
  - Lag phase
  - Log phase
  - Stationary phase
  - Phase of decline

Lag phase



- Period between inoculation and beginning of multiplication of bacteria.
- Bacteria increase in size due to accumulation of enzymes and metabolites.
- Bacteria reach their maximum size at the end of lag phase.

Log phase

- Bacteria divide exponentially so that the growth curve takes a shape of straight line. At this stage, the bacterium is-
  - Smaller in size
  - Biochemically active- It is the best stage to perform the biochemical reactions
  - Uniformly stained- It is the best time to perform the Gram stain

Stationary phase

- Number of viable cells remains stationary as there is almost a balance between the dying cells and the newly formed cells.
  - Bacterium becomes Gram variable
  - More storage granules are formed
  - Sporulation occurs in this phase
  - Bacteria produce exotoxins, antibiotics and bacteriocins

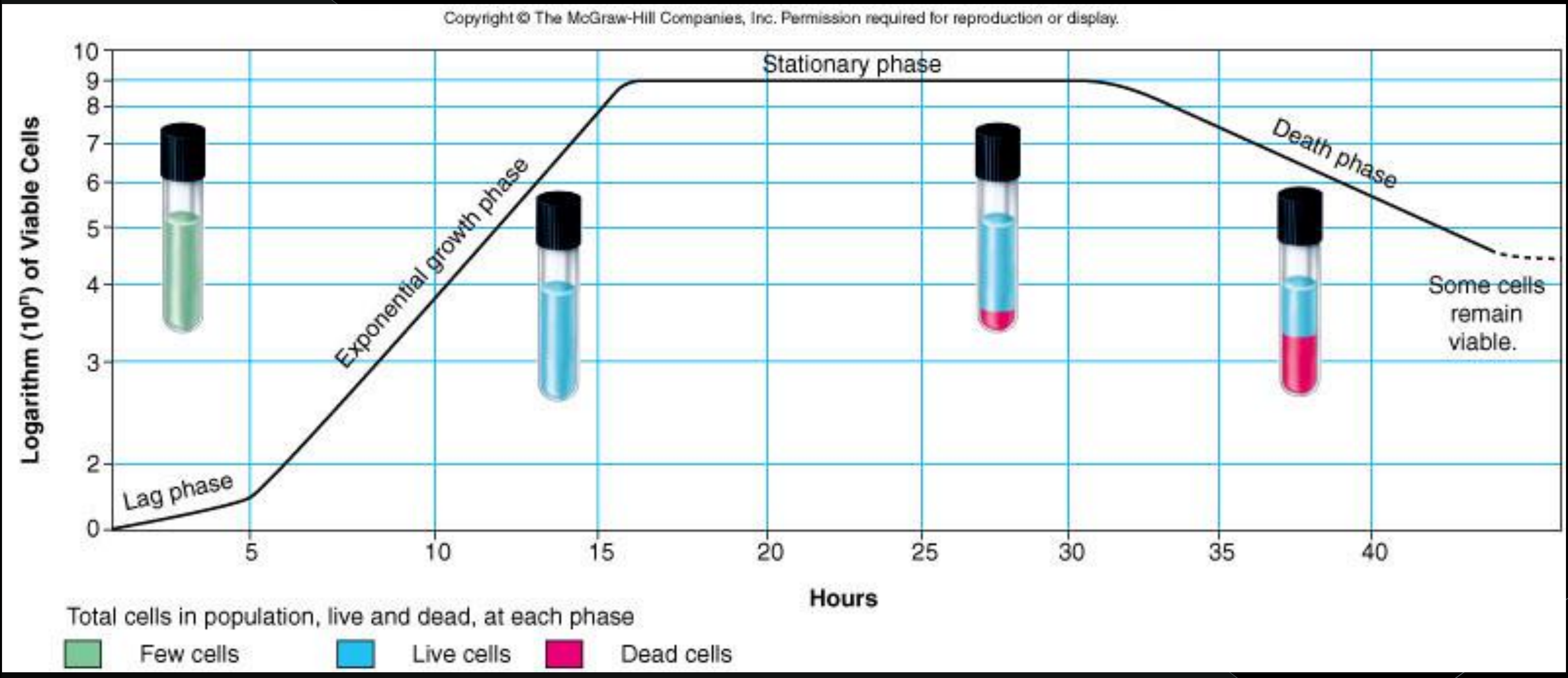
Decline phase

- Bacteria stop dividing completely; while the cell death continues due to exhaustion of nutrients, and accumulation of toxic products.

Bacterial growth curve

	Lag	Log	Stationary	Decline
Bacteria divide	No	Yes	Yes	No
Bacterial death	No	No	Yes	Yes
Total count	Flat	Raises	Raises	Flat
Viable count	Flat	Raises	Flat	Falls
Special features	Accumulation of enzymes & metabolites Attains max. size	Uniformly stained, Metabolically active Small size	Gram variable Produce- Granules Spores Exotoxin, Antibiotics Bacteriocin	Produce involution forms

Standard Growth Curve



Thank you