Microbial Growth and Nutritional Requirements

Microbial Growth:

- It refers to an increase in **cell number**, not in cell size.
- Bacteria grow and divide by **binary fission**, a rapid and relatively simple process.

Requirements for Growth : Bacterial growth requirements can be categorized as Physical, Chemical requirements.

I Physical Requirements

1.Temperature:

2. pH

3. Ionic concentration (Osmotic pressure)

1. Temperature :

Microbes are classified into several groups based on their preferred temperature ranges.

A. Psychrophiles: "Cold-loving". Can grow at 0°C. Can be classified as

1.True Psychrophiles: Sensitive to temperatures over 20°C. Optimum growth at 15°C or below. Found in very cold environments (North pole, ocean depths). Seldom cause disease or food spoilage.

2.Psychrotrophs: Optimum growth at 20 to 30°C. Responsible for most low temperature food spoilage.

B. Mesophiles: "Middle loving". Most bacteria comes under this category. Include most pathogens and common spoilage organisms. These bacteria grow best between 25 to 40°C. Optimum temperature is commonly 37°C. Many have adapted to live in the bodies of animals

C. Thermophiles: "Heat loving". The optimum growth is between 50 to 60°C. Many cannot grow below 45°C. They are adapted to live in sunlit soil, compost piles, and hot springs. Some thermophiles form extremely heat resistant endospores. Bacteria that have optimum growth at 80°C or higher are called **Hyperthermophiles** or **Extreme Thermophiles**. Example-Archaebacteria. Most live in volcanic and ocean vents.

2. pH:

Most **bacteria** prefer neutral pH (6.5-7.5). **Molds** and **yeast** grow in wider pH range, but prefer pH between 5 and 6. **Acidity** inhibits most microbial growth and is used frequently for food preservation (e.g.: pickling). **Alkalinity** inhibits microbial growth, but not commonly used for food preservation. Acidic products of bacterial metabolism interfere with growth. Buffers can be used to stabilize pH. Based on pH requirements, organisms can be classified as:

A. **Acidophiles**: "Acid loving".

- Grow at very low pH (0.1 to 5.4)

- Example *Lactobacillus* - produces lactic acid, tolerates mild acidity.

B. **Neutrophiles**:

- Grow at pH 5.4 to 8.5. Includes most human pathogens.

3. Osmotic Pressure :

Cells are 80 to 90% made up of water. Hence hypertonic solutions cause high osmotic pressure inside the cell and removes water from cell, causing shrinkage of cell membrane (plasmolysis). This process is used to control spoilage and microbial growth. Example - Sugar in jelly and Salt on meat. Like that hypotonic solutions cause low osmotic pressure and water to enter into the cell. Microbe may lyse or burst if cell wall is weak. Hence Bacteria requires an optimum ionic concentration that causes the osmotic pressure. Based on this bacteria are classified as

a. **Halophiles** : Require moderate to large salt concentrations. As ocean water contains 3.5% salt, most bacteria in oceans are halophiles.

b. **Extreme or Obligate Halophiles**: Require very high salt concentrations (20 to 30%).

- Bacteria in Dead Sea, brine vats.

c. **Facultative Halophiles**: Do not require high salt concentrations for growth, but tolerate 2% salt or more.

II Chemical Requirements

1. Carbon : 50% of dry weight of cell is made up of carbon which is the structural backbone of all organic compounds. Bacteria are classified into two groups based on how they obtain carbon.

i. Chemoheterotrophs : Obtain carbon from lipids, proteins, and carbohydrates in the media.

ii. Chemoautotrophs and Photoautotrophs : Obtain carbon directly from carbon dioxide.

2. Nitrogen: Makes up 14% of dry cell weight. Used to form amino acids, DNA, and RNA.

Sources of nitrogen are – i) **Protein** for most bacteria, ii) **Ammonium** that is found in organic matter, iii) **Nitrogen gas (N_2) that is** obtained directly from atmosphere. Important **nitrogen fixing bacteria** that live free in soil or associated with legumes (peas, beans, alfalfa, clover, etc.) are few examples. The another source is iv) **Nitrates**: Salts that dissociate to give NO_3.

3. Sulfur: Used to form proteins and some vitamins (thiamin and biotin). **Sources of sulfur** are protein for Most bacteria, Hydrogen sulfide and **Sulfates**: Salts that dissociate to give SO

4. Phosphorus: Used to form DNA, RNA, ATP, and phospholipids. The s**ources** are mainly inorganic phosphate salts and buffers.

5. Other Elements: Potassium, magnesium, and

calcium are often required as enzyme cofactors. **Calcium** is required for cell wall synthesis in Gram positive bacteria.

6. **Trace Elements:** .Many are used as enzyme cofactors. Commonly found in tap water.
    a. Iron, b. Copper, c. Molybdenum d. Zinc

7. **Oxygen :** Organisms that use molecular oxygen (O_2), produce more energy from nutrients than anaerobes. We can classify microorganism based on their oxygen requirements:

A. Obligate Aerobes : Require oxygen to live.

Disadvantage : Oxygen dissolves poorly in water.

Example: *Pseudomonas*, a common nosocomial pathogen.

B. Facultative Anaerobes: Can use oxygen, but can grow in its absence. Have complex set of enzymes. Examples: *E. coli, Staphylococcus*, yeasts, and many intestinal bacteria.

C. Obligate Anaerobes: Cannot use oxygen and are harmed by the presence of toxic forms of oxygen.
Examples: *Clostridium* bacteria that cause tetanus and botulism.

D. Aerotolerant Anaerobes: Can't use oxygen, but tolerate its presence. Can break down toxic forms of oxygen.

Example: *Lactobacillus* carries out fermentation regardless of oxygen presence.

E. Microaerophiles: Require oxygen, but at low concentrations. Sensitive to toxic forms of oxygen.

Example: *Campylobacter*.

Nutritional media and Growth conditions;

Culture Medium: Nutrient material prepared for microbial growth in the laboratory.

Culture: Microbes that grow and multiply in or on a culture medium.

Requirements:

- ✓ Must be sterile
- ✓ Contain appropriate nutrients with minimal requirements
- ✓ Must be incubated at appropriate growth conditions like temperature, pH and oxygen

Minimal Requirements

Every microbe has its own specific minimal nutritional requirement. If it is not provided, they do not grow. This minimal requirement consists of a carbon source, nitrogen source, sulphur source. phosphorus source besides energy source. They grow better in the presence of particular amino acids or vitamins or other compounds, so that the species could grow or develop better.

Carbon source (glucose etc.) is essential for the basic cell structure because each and every biomolecule is made up of carbon along with other compounds.

Nitrogen source is required for the biosynthesis of amino acids, nucleic acids, enzymes etc.

Sulphur and phosphorous required for synthesizing nucleic acids, vitamins, and certain amino acids.

A photosynthetic microorganism eg. Cyanobacteria do not require an energy source. They use sunlight and trap the form of chemical energy, used frequently. With the help of CO_2 and water, they synthesize food in the form of carbohydrate. But many microorganisms need some energy. Culture media provides all these minimal requirements of energy sources.

Types of Culture Media

I Classification according to Consistency:

1. Solid Media - with 1.5 to 3.0% agar e.g Nutrient Agar
2. Liquid Media - no solidifying agent e.g. Nutrient Broth
3. Semi solid Media - with less than 1.5% agar e.g. SIM (Sulfide Indole Motility Medium)

II. Classification According to composition:

1. **Chemically Defined Media:** Nutrient material whose exact chemical composition is known.

   It is not widely used and is expensive.

2. **Complex Media:** Nutrient material whose exact chemical composition is **not known.**

- ✓ Widely used for heterotrophic bacteria and fungi.
- ✓ Made of extracts and digests from yeast, meat, plants, protein digests, etc.
- ✓ Composition may vary slightly from batch to batch.
- ✓ There are two forms of complex media:

a) Nutrient agar: it isnanSolid culture media. Nutrient Agar is used as a general-purpose medium for the growth of a wide variety of non-fastidious microorganisms. It consists of peptone, beef extract, and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of non-fastidious microorganisms.

Composition of nutrient Agar

Agar	- 1.5 %
Peptone	- 0.5 %
Beef extract	- 0.3%
Sodium chloride	- 0.5%

pH 7.4 ± 0.2 at 25°C

b) Nutrient broth: it is a Liquid media. Nutrient broth contains same ingredients except agar. Nutrient Agar/broth is used for the cultivation and maintenance of non-fastidious organisms as well as enumeration of organisms in water, sewage, dairy products, feces and other materials.

Characteristics of the components used in nutrient agar/broth

1. Beef extract is an aqueous extract of lean beef tissues. It contains water-soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water soluble vitamins, and salts.
2. Peptone is made by digesting proteinaceous materials e.g., meat, casein, gelatin, using acids or enzymes. Peptone is the principal source of organic nitrogen and may contain carbohydrates or vitamins. Depending up on the nature of protein and method of digestion, peptones differ in their constituents, differing in their ability to support the growth of bacteria.
3. Agar is a complex carbohydrate obtained from certain marine red algae. It is used as a solidifying agent for media and does not have any nutritive value. First used by Robert Koch.

Unique Properties of Agar:

✓ Melts above 95^{o}C.

✓ Once melted, does not solidify until it reaches 40^{o}C.

✓ Cannot be degraded by most bacteria.

✓ Originally used as food thickener.

III Classification according to Function/Application:

1. Anaerobic Growth Media: Used to grow anaerobes that might be killed by oxygen.

It Contains ingredients that chemically combine with oxygen and remove it from the medium.

Example: Sodium thioglycolate

✓ Tubes are heated shortly before use to drive off oxygen.

✓ Plates must be grown in oxygen free containers (anaerobic chambers).

2. Selective Media: Used to suppress the growth of unwanted bacteria and encourage the growth of desired microbes.

✓ Saboraud's Dextrose Agar: pH of 5.6 discourages bacterial growth. Used to isolate fungi.

✓ Brilliant Green Agar: Green dye selectively inhibits gram-positive bacteria. Used to isolate gram-negative *Salmonella*.

✓ Bismuth Sulfite Agar: Used to isolate *Salmonella typhi*. Inhibits growth of most other bacteria.

3. Differential Media: Used to ***distinguish*** colonies of a desired organism.

✓ **Blood Agar:** Used to distinguish bacteria that destroy red blood cells (**hemolysis**).

Hemolysis appears as an area of clearing around colony.

Example: *Streptococcus pyogenes*.

▶ **Both Selective and Differential Media:** Used both to ***distinguish*** colonies of a desired organism, and ***inhibit*** the growth of other microbes.

✓ **Mannitol Salt Agar:** Used to distinguish and select for *Staphylococcus aureus*.

- ▶ High salt (7.5% NaCl) discourages growth of other organisms.
- ▶ pH indicator changes color when mannitol is fermented to acid.

✓ **MacConkey Agar:** Used to distinguish and select for *Salmonella*.

- ▶ Bile salts and crystal violet discourage growth of gram- positive bacteria.
- ▶ Lactose plus pH indicator: Lactose fermenters produce pink or red colonies, nonfermenters are colorless.

4. Enrichment media: Used to favour the growth of a microbe that may be found in very small numbers. Unlike selective medium, does not necessarily suppress the growth of other microbes. Used mainly for fecal and soil samples. After incubation in enrichment medium, greater numbers of the organisms can be obtained.

5. Special Culture Media: Used to grow bacteria with unusual growth requirements.

I. **Bacteria that do not grow on artificial media**:

a. *Mycobacterium leprae* (leprosy): Grown in armadillos.

b. *Treponema pallidum* (syphilis): Grown in rabbit testicles.

c. Obligate intracellular bacteria (rickettsias and chlamydias): Only grow in host cells.

II. **Bacteria that require high or low CO_2 levels:**

a. **Capnophiles**: Grow better at high CO_2 levels and low O_2 levels. Similar to environment of intestinal tract, respiratory tract, and other tissues.

CULTURE METHODS OF MICROORGANISMS

Culture methods are very crucial in a microbiology laboratory. Various culture methods are carried out to:

1. Isolate bacteria in pure culture and identify the same by performing various tests.
2. Demonstrate biochemical, antigenic, and other phenotypic and genomic properties of the isolated colonies.
3. Demonstrate susceptibility of the isolated bacteria to antibiotics, bacteriophages, bacteriocins, etc.
4. Prepare antigens for various uses.
5. Maintain stock culture.

STEPS IN CULTURING BACTERIA

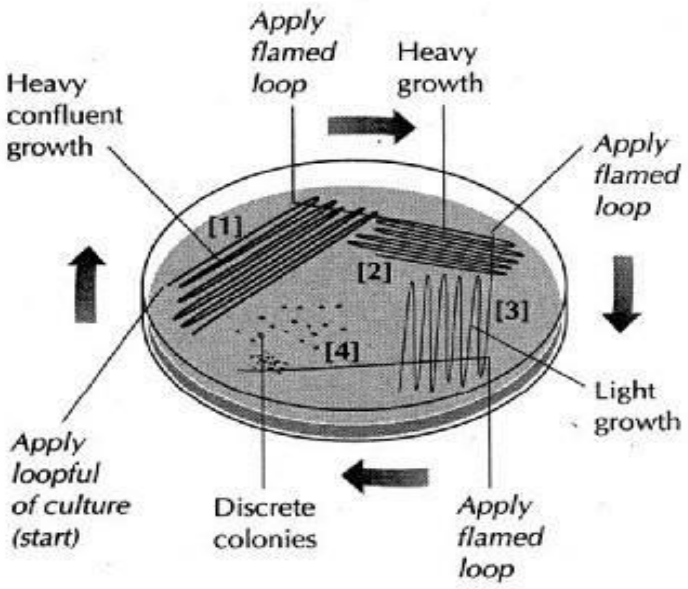
1. Selection and Preparation of media
2. Sterilization
3. Inoculation
4. Culture
5. Isolation of pure culture
6. Demonstrate susceptibility of the isolated bacteria to antibiotics, bacteriophages, bacteriocins, etc.
7. Prepare antigens for various uses.
8. Maintain stock culture.

METHODS OF CULTURE

Various methods are used for culturing of bacteria. These include (1.) streak culture, (2) lawn culture, (3) pour-plate culture, (4) stroke culture, (5) stab culture, and (6) liquid culture.

1.Streak Culture

- Streak culture is the most useful method for obtaining discrete colonies of the bacteria.
- It is carried out by streaking on the surface of a solid media plate using a platinum or nichrome loop of 2–4 mm diameter.
- In this method, a loopful of the inoculum is placed near the peripheral area of the plate.
- The inoculum is then spread with the loop to about one-fourth of the plate with close parallel strokes.
- From the primary inoculum, it is spread thinly over the plate by streaking with the loop in parallel lines.
- The loop is flamed and cooled in between the streaks to obtain isolated colonies.
- The inoculated culture plate is incubated at 37°C overnight for demonstration of colonies.
- Confluent growth occurs at the primary inoculum, but becomes progressively thinner, and well-separated colonies are demonstrated on the final streaks of the inoculum
- Single isolated colonies obtained by this method are very useful to study various properties of bacteria.



2. Lawn Culture

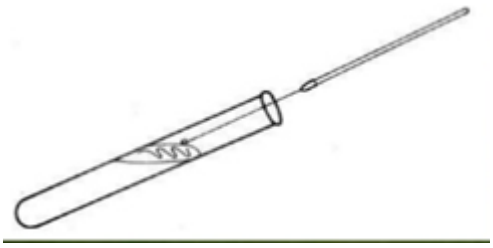
- The lawn culture provides a uniform layer of bacterial growth on a solid medium.
- It is carried out by flooding the surface of the solid media plate with a liquid culture or suspension of bacteria, pipetting off the excess inoculum, and finally incubating the plate overnight at 37°C.
- Alternatively, the culture plate may be inoculated by a sterile swab soaked in liquid bacterial culture or suspension and incubating overnight for demonstration of the bacterial colonies.

3. **Pour-Plate Culture**

- The pour-plate culture is used to determine approximate number of viable organisms in liquids, such as water or urine.
- It is used to quantitate bacteria in urine cultures and also to estimate the viable bacterial count in a suspension.
- This method is carried out in tubes, each containing 15 mL of molten agar.
- The molten agar in tubes is left to cool in a water bath at 45°C.
- The inoculum to be tested is diluted in serial dilution. Then 1 mL each of diluted inoculum is added to each tube of molten agar and mixed well.
- The contents of tubes are poured into sterile Petri dishes and allowed to set.
- After overnight incubation of these Petri dishes at 37°C, colonies are found to be distributed throughout the depth of the medium, which can be counted using a colony counter.

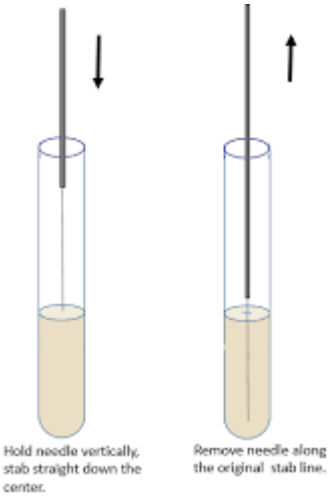
4. Stroke Culture

Stroke culture provides a pure growth of bacteria for carrying out slide agglutination and other diagnostic tests. It is carried out in tubes usually containing nutrient agar slopes.



5. Stab Culture

- Stab culture is prepared by stabbing the medium in tubes with a long, straight wire and incubating at 37°C. It is used for demonstration of gelatin liquefaction, oxygen requirements of the bacterium under study and for maintenance of stock cultures.



6. Liquid Culture

- Liquid culture is prepared in a liquid media enclosed in tubes, flasks, or bottles.
- The medium is inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes and incubating at 37°C, followed by subculture on to solid media for final identification.
- A major disadvantage of liquid culture is that it does not provide pure culture of the bacteria and also the bacterial growth does not exhibit special characteristic appearances.

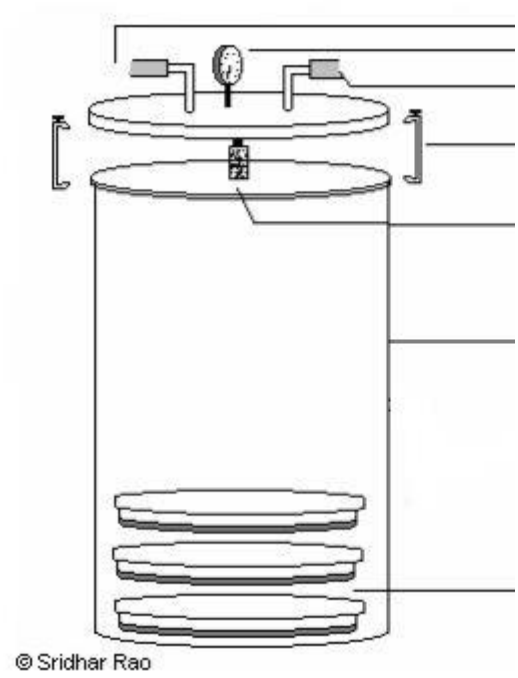
7. Anaerobic Culture

- Obligate anaerobes are bacteria that can live only in the absence of oxygen. These anaerobes are killed when exposed to the atmosphere for as briefly as 10 minutes.
- Anaerobes are tolerant to small amounts of oxygen. Facultative anaerobes are those anaerobes that grow with or without oxygen.
- Anaerobic cultures are carried out in an environment that is free of oxygen, followed by incubation at 95°F (35°C) for at least 48 hours before the plates are examined for growth.
- The cultures of anaerobic bacteria are carried out as follows:

1. Producing a vacuum
2. Oxygen displacement
3. Oxygen absorption
4. Reducing agents
5. Anaerobic chambers–(have catalyst, desiccant, H2, CO2, N2 + indicator; airtight gloves) . example McIntosft–Fildes anaerobic jar and Gaspak system

1. **McIntosft–Fildes anaerobic jar**:
   McIntosh and Fildes' anaerobic jar is an instrument used in Microbiology laboratory, for the generation of anaerobic condition (anaerobiosis) to culture obligates anaerobes such as Clostridium spp.

- Anaerobiosis obtained by McIntosh and Fildes' anaerobic jar is one of the excellent and most widely used method for anaerobiosis but it requires costly special apparatus and vacuum pump.



- McIntosft–Fildes anaerobic jar consists of a glass or metal jar with a metal lid that can be clamped air tight with the help of a screw.
- The lid has one inlet tube and another outlet tube.
- The outlet tube is connected to a vacuum pump by which the air is evacuated out of the jar.
- The inlet tube is connected to a source of hydrogen supply.
- The lid has two electric terminals also that can be connected to an electric supply.
- The underside of the lid contains a catalyst (e.g., alumina pellets coated with palladium) that catalyzes the union of hydrogen with residual oxygen present in the air.
- This method ensures complete anaerobiosis.

Principle:

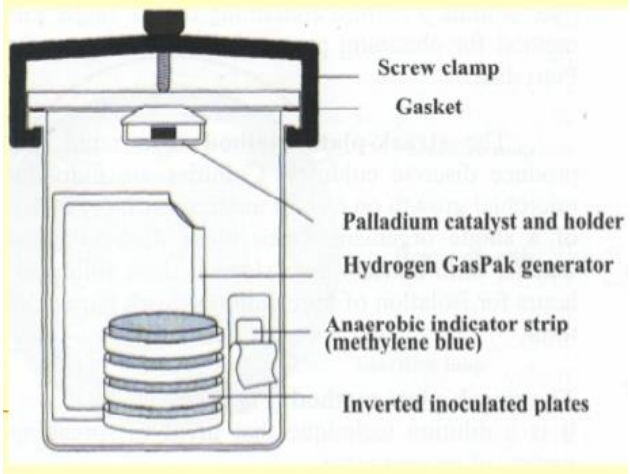
- McIntosh and Fildes' anaerobic jar works on the principle of evacuation and replacement, where the air inside the chamber is evacuated and replaced with mixture of gases (consisting of 5%CO2, 10%H2 and 85%N2) .
- It is practically impossible to evacuate all the air so some amount of oxygen will still be left behind.
- The residual oxygen left behind is converted to water using Spongy palladium or platinum catalyst. The catalyst acts as a catalyzing agent causing slow combination of hydrogen and oxygen to form water.
- Reduced methylene blue is generally used as indicator (mixture of NaOH, methylene blue, and glucose). It becomes colorless anaerobically but regains blue color on exposure to oxygen.

2. Gas pack system

- Gas pack system is used to create an oxygen-free environment for the growth of anaerobic microorganisms. At present, it is the most commonly used method for anaerobiosis. It is very simple to perform

Components of GasPak Anaerobic System

1. polycarbonate jar (or anaerobic container)
2. a lid with a gasket to prevent airflow (for jar system)
3. Indicator strip (a strip impregnated with an oxidation-reduction indicator such as methylene blue or resazurin)
4. disposable gas generating pouch (a pouch containing sodium borohydride and sodium bicarbonate)
5. a palladium catalyst



Principle

- Inoculated plates or tubes are placed inside the polycarbonate jar or anaerobic container along with gas generator sachet and indicator strip and is sealed completely.
- In the presence of water, chemicals present inside the sachet i.e. sodium bicarbonate ($NaHCO_3$) and sodium borohydride ($NaBH_4$) react chemically producing hydrogen and carbon dioxide gas.
- The hydrogen thus produced reacts with oxygen present inside the jar producing water (which forms as condensation on the inside of the jar).
- $2H_2 + O_2 + \text{catalyst} = 2H_2O$
- This reaction is catalyzed by the element palladium, which is attached to the underside of the lid of the jar. The carbon dioxide replaces the removed oxygen, creating a completely anaerobic environment.

METHODS OF ISOLATING PURE CULTURES

A pure culture theoretically contains a single bacterial species. There are a number of procedures available for the isolation of pure cultures from mixed populations. The following methods may be employed for isolating pure cultures of bacteria from mixtures:

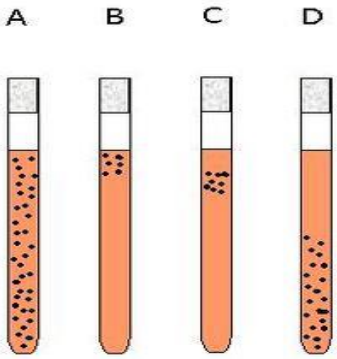
1. **Surface plating**

This is the method routinely employed in clinical bacteriology and enables the isolation of distinct colonies which may be picked out, if necessary, for further purification and study.

2. **Enrichment, selective and indicator media** are widely used for the isolation of pathogens from specimens such as feces, with varied flora.
3. **Pre-treatment of specimens :**

Pure cultures may be obtained by pre-treatment of specimens with appropriate bactericidal substances which destroy the unwanted bacteria. This method is the standard practice for the isolation of tubercle bacilli from sputum and other clinical specimens, by treatment with alkali, acid or other substances to which most commensals are susceptible but tubercle bacilli are resistant.

4. **Obligate aerobes and anaerobes** may be separated by cultivation under aerobic or anaerobic conditions. Shake cultures in Veillon tubes were in use formerly but are now obsolete. This consists of a glass tube open at both ends. One end is closed with a rubber stopper and molten glucose agar in which the inoculum is evenly dispersed is poured into the tube and allowed to set in a vertical position. The top of the tube is closed with a cotton plug. On incubation, the bacteria in the inoculum differentiate depending on their oxygen requirement.



The obligate aerobes grow at the top and the anaerobes at the bottom, while the facultative bacteria grow throughout the column. The entire medium can be extruded on to a plate and the different colonies fished out.

5. **Incubation at different temperatures**:

   Separation of bacteria with different temperature optima can be effected by incubation at different temperatures. Only thermophilic bacteria grow at 60°C. A mixture containing N. *meningitidi*s and N. cat*arrhalis* can be purified by incubation at 22 °C when only the latter grows.

6. **By heating** a mixture containing vegetative and spore forming bacteria, at 80°C the former can be eliminated. This method is useful for the isolation of tetanus bacilli from dust and similar sources.

7. **Separation of motile from nonmotile bacteria** can be effected using *Craigi*e's *tube.* This consists of a tube of semisolid agar, with a narrow tube open at both ends placed in the centre of the medium in such a way that it projects above the level of the medium. The mixture is inoculated into the central tube. On incubation, the motile bacteria alone traverse the agar and appear at the top of the medium outside the central tube. AU-tube also serves the same purpose, inoculation being performed in one limb and the subculture taken from the other. This method can also be used to obtain phase variants in Salmonella species.

8. **Pathogenic bacteria** may be isolated from mixtures by inoculation into appropriate animals. Anthrax bacilli can be distinguished from other aerobic sporulating bacilli by inoculation into mice or guinea pigs. Anthrax bacilli produce a fatal septicaemia and may be cultured pure from the heart blood.

9. **Bacteria of differing sizes** may be separated by the use of selective filters. Filters are widely used for separating viruses from bacteria.

Microbial growth

Growth

Increase in the quantity of cellular components and structure (mass) or cell number (population)

- The growth of an individual cell is hard to monitor because of the size, so we focus on the growth of populations. Individual cells also don't affect the environment as much.
- Prokaryotes generally have 2 types of growth
    - Binary fission
        - Cell components partitioned equally among 2 daughter cells
        - Most bacteria, unicellular algae, and protozoa
    - Budding
        - Newly synthesized components appear only in daughter cells
        - Daughter grows off the parent cell and the parent can form many daughters
        - Yeast and other fungi are good examples of these

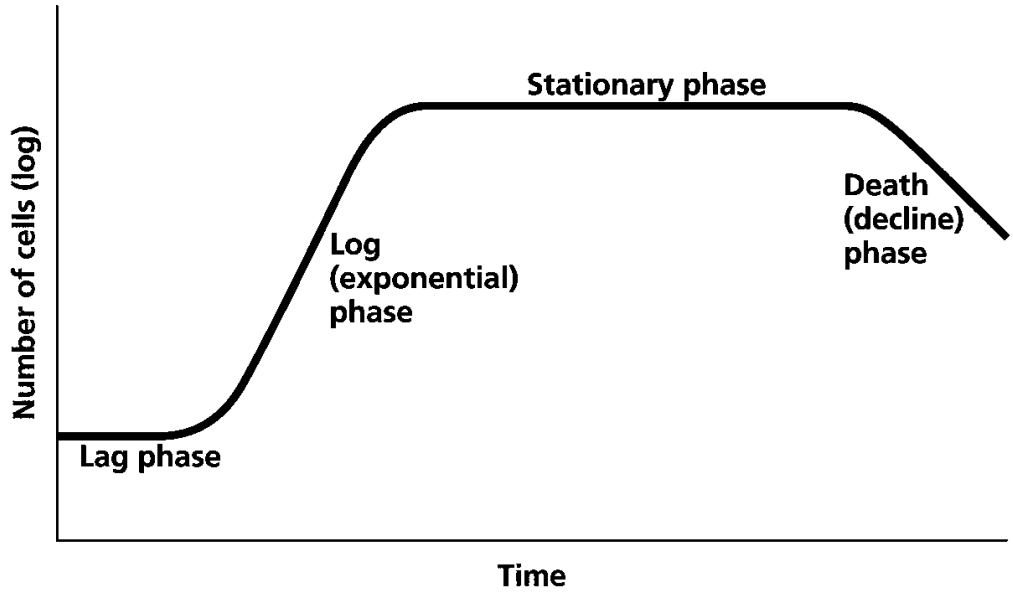
Bacterial cell cycle

- The period in which newly formed bacteria replicate DNA, elongate and then divide
- Under optimum conditions, cells divide every 20 minutes, but replication of DNA takes 40 minutes, this is because DNA never stops replicating

Population growth

- Growth rate - change in cell number/mass per unit time
- Generation - interval for formation of 2 cells from 1
- Generation doubling time - the time for one generation to form
- Under appropriate conditions, bacteria grow with constant generation time
- *E. coli* will always take approximately 20 minutes to divideThis allows us to plan experiments in labs and when we talk about food safety in terms of food poisoning

The phases of bacterial cell life



When bacteria are inoculated from a slant culture into a known volume of liquid medium , the population undergoes a characteristic sequence in its rate of increase in cell number. If the cell population is counted at intervals, it is possible to plot a typical bacterial growth curve that shows the growth of cells over time. It shows four distinct phases of growth.

- **Lag phase:** Slow growth or lack of growth due to physiological adaptation of cells to culture conditions or dilution of exoenzymes due to initial low cell densities. There is no increase in number of viable cells. During this phase the cells increase in size. but there is no cell division. Actually, the cells prepare for cell division. The length of the lag phase depends on many factors like age of the inoculum, the composition of the growth medium and environmental factors such as temperature, pH, aeration etc.,. The lag phase is followed by log phase.

- **Log or exponential phase:** Optimal growth rates, during which cell numbers double at discrete time intervals known as the mean generation time. (Exponential growth - total cell number doubles with each generation – like 2,4,8,16,32,64 …….(2^1, 2^2,2^3 ,2^4,…….2 ^{n}). The cells divide at a maximum rate permitted by the composition of the medium and environmental conditions. The phase represents a population of cells at different age. The population is not homogenous. The log phase is followed by the stationary phase.

- **Stationary phase:** Growth (cell division) and death of cells counterbalance each other resulting in no net increase in cell numbers. The reduced growth rate is usually due to a lack of nutrients and/or a build up of toxic waste constituents. It is followed by a decline phase.

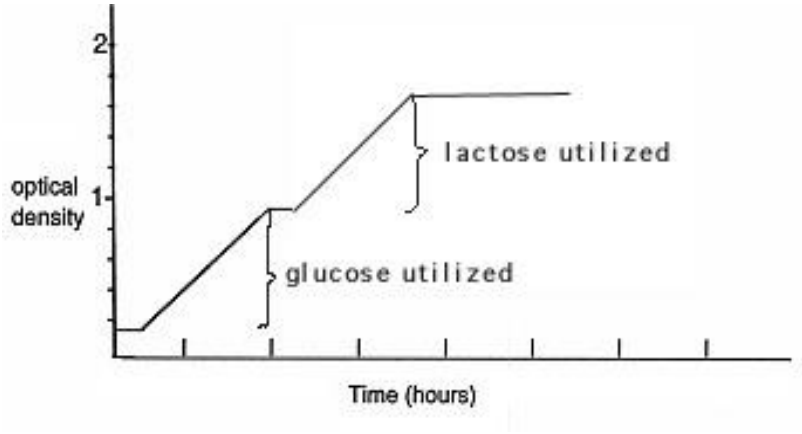
- **Decline or death phase:** charecterised by an exponential decrease in number of viable cells. Death rate exceeds growth rate resulting in a net loss of viable cells. When a population enters decline phase they have to be re inoculated in a fresh medium. When do so, they will not continue to die, but start lag phase. But cells from decline phase usually have a long lag phase than cells from log phase or stationary phase.

Synchronous Growth

To study the behaviour and properties of cells in culture, a synchronous culture is created with cells uniformly at division cycle and physiologically identical. A synchronous culture is either generated by physically sepeatating the cells at same stage of division or by forcing a population to attain an identical physiological condition by a change in environment.

Diauxic Growth

In a culture medium containing two carbon sources, bacteria such as *E.coli* displays a growth curve, called diauxic.



Under this condition, if both glucose and lactose are supplemented in medium having *E.coli*, first *E.coli* will utilize glucose and after it is exhausted lactose will be utilized. In between, there is a short lag period. This type of growth curve is called diauxic growth curve.