Microbial Growth & Nutritional requirements

1st Course

Lec.#4

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- In microbiology, **growth** is defined as an increase in the number of cells rather than in the size of individual cells.
- **Bacterial growth** is asexual reproduction, or cell division, of a bacterium into two daughter cells, in a process called **binary fission**. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell.

  and the time required for this process is called the **generation time.**

- **Generation Time (Doubling time)**

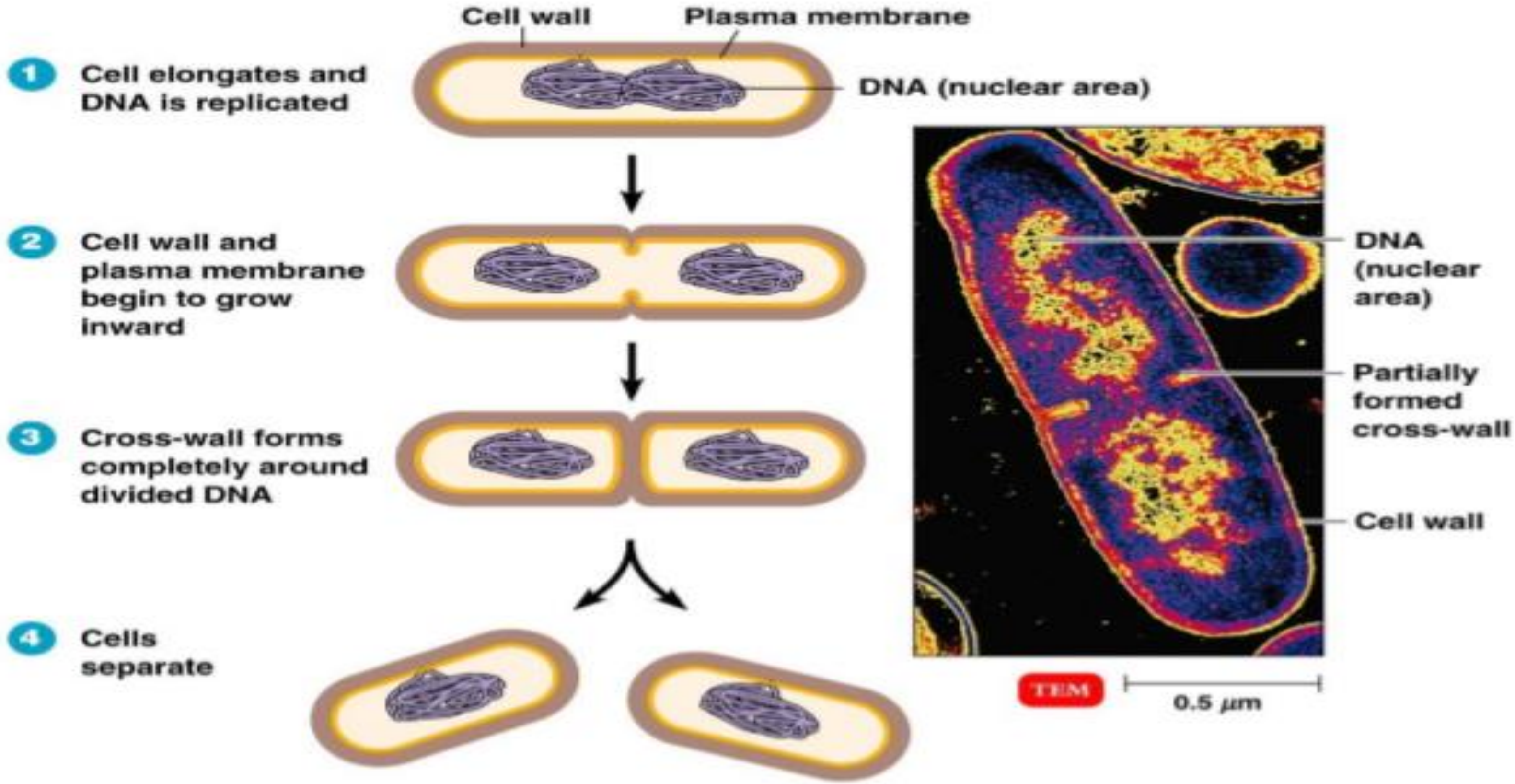
The time required for a cell to divide (and its population to double)

Cell's division produces two cells, two cells' divisions produce four cells, and so on.

examples';

- Escherichia coli 20 minutes –
- Mycobacterium tuberculosis 18 hours
- Mycobacterium leprae 14 days

Bacterial Growth



(a) A diagram of the sequence of cell division.

(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

Standard Growth Curve

1. Lag Phase

- When a microbial culture is inoculated into a fresh medium, growth usually begins only after a period of time called the lag phase.
- This interval may be brief or extended, depending on the history of the inoculum and the growth conditions.
- making new enzymes in response to new medium.
- The length of lag phase depend upon:

a. Type of bacteria.

b. Better the medium, shorter the lag phase.

c. The phase of culture from which inoculation is taken.

d. Size or volume of inoculum.

e. Environmental factors like temperature.

2. Log phase – Logarithmic (Exponential) phase

In logarithmic phase the bacterial cell start dividing and their number increase by geometric progression with time (each cell divides to form two cells, each of which also divides to form two more cells, and so on) depending on the available resources and other factors.. During this period.

a. Cells in exponential growth are typically in their healthiest state .

b. Bacteria have high rate of metabolism

c. Bacteria are more sensitive to antibiotics and radiation .

d. Rates of exponential growth vary greatly. The rate of exponential growth is influenced by environmental conditions (temperature, composition of the culture medium), as well as by genetic characteristics of the organism itself.

In general, prokaryotes grow faster than eukaryotic microorganisms, and small eukaryotes grow faster than large ones.

3. Stationary Phase

In a batch culture (tube, flask bottle, Petri dish), exponential growth is limited. It may be due to:

a. An essential nutrient of the culture medium is used ups.

b. Accumulation of toxic products(a waste product) in the medium and sporulation may occur during this stage.

In stationary phase rate of multiplication and death becomes almost equal (death rate = division rate) .

In the stationary phase, there is no net increase or decrease in cell number and thus the growth rate of the population is zero.

4. Death Phase or Decline phase

- If incubation continues after a population reaches the stationary phase, the cells may remain alive and continue to metabolize, but they will eventually die. When this occurs, the population enters the death phase of the growth cycle. In some cases death is accompanied by actual cell lysis.

The factors responsible are:

a. Nutritional exhaustion

b. Toxic accumulation

c. Autolysin enzymes

- **The phases of bacterial growth are reflections of the events in a population of cells, not in individual cells.**

Standard Growth Curve

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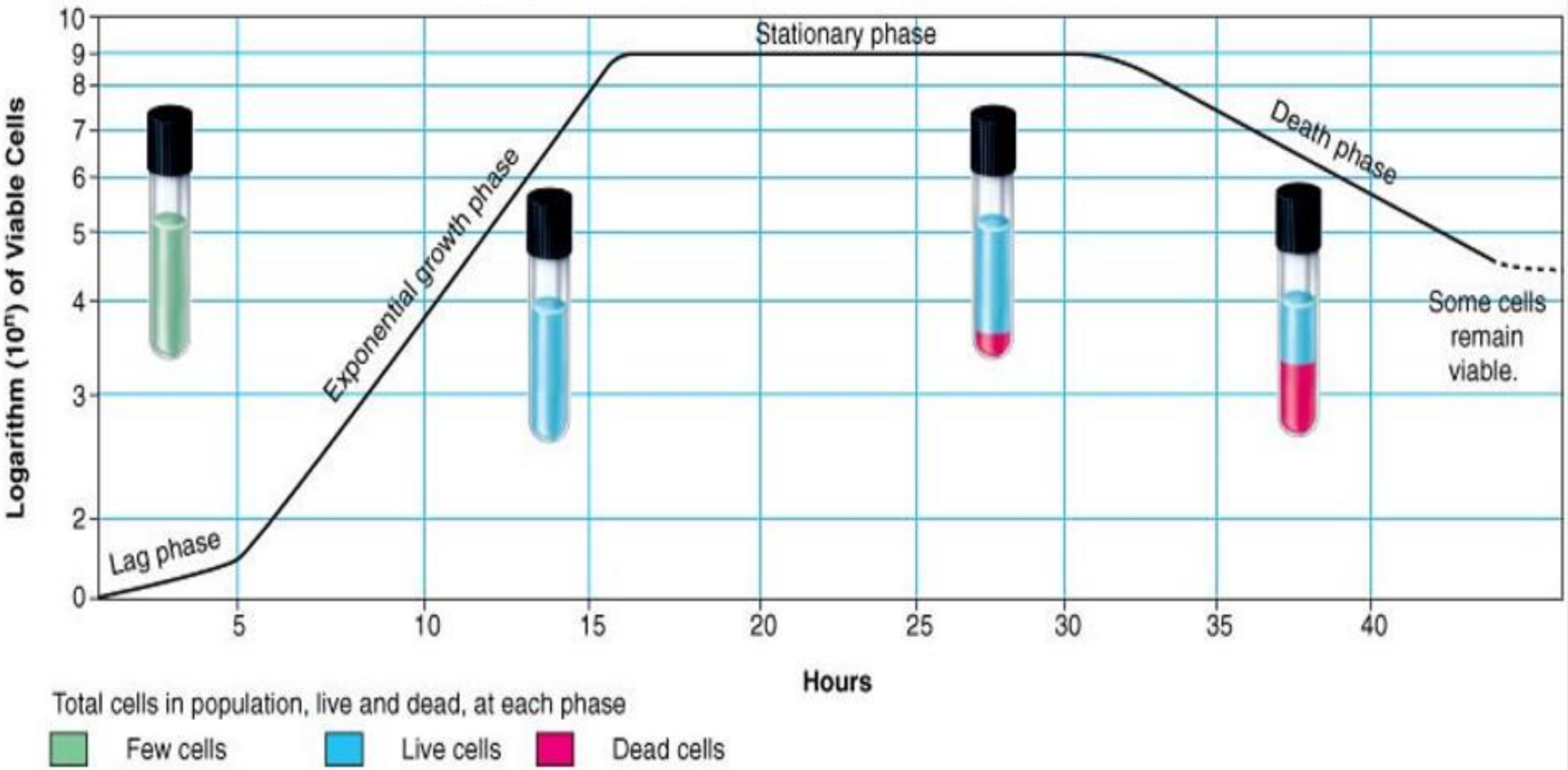


Measurement of Cell Numbers

There are two types :

1- Direct cell counts:

a- Counting chambers

b- Electronic counters

c- Membrane filters

d- Dilution series ; viable cell counts
plating methods (spread, pour plate).

2.Indirect cell counts:

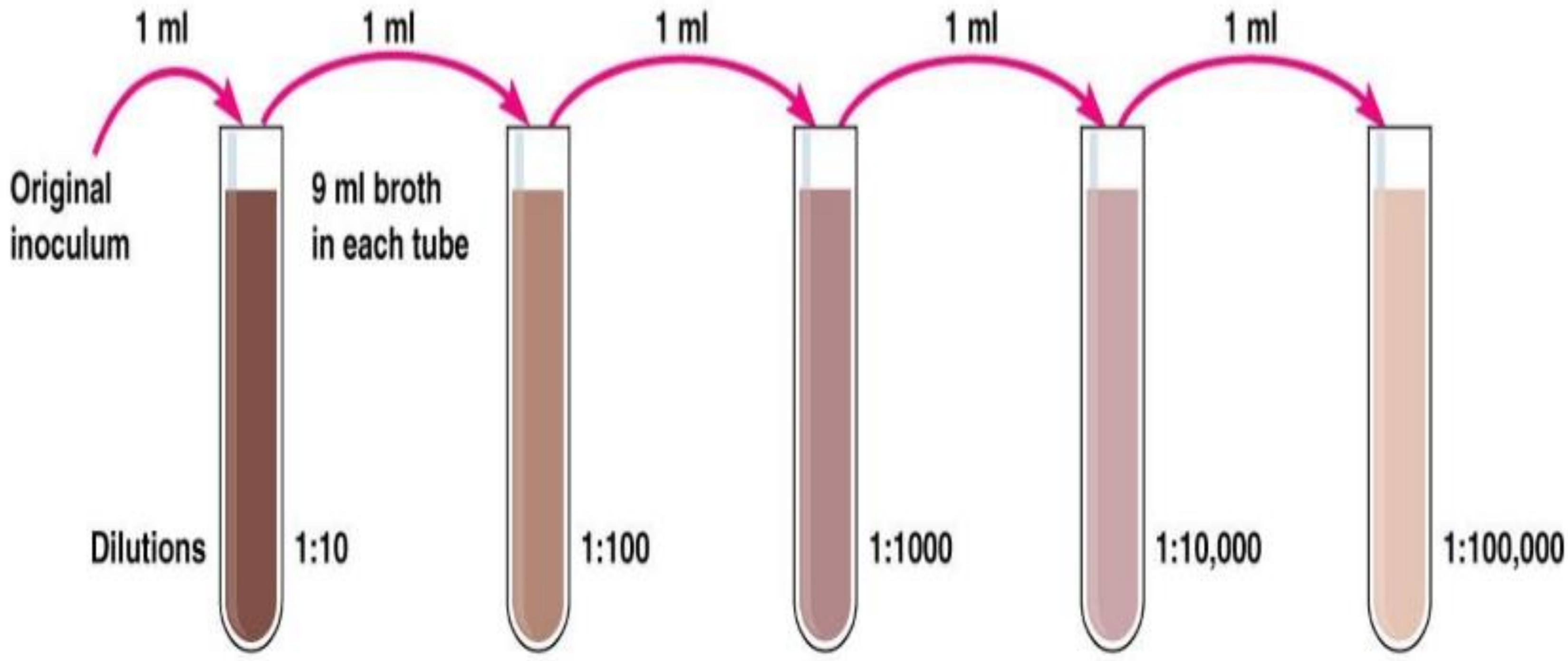
a- Optical density

b- Dry weight of culture

c- Metabolic activity(chemicals)

Serial Dilutions

Direct Measurements of Microbial Growth Plate counts: Perform serial dilutions of a sample

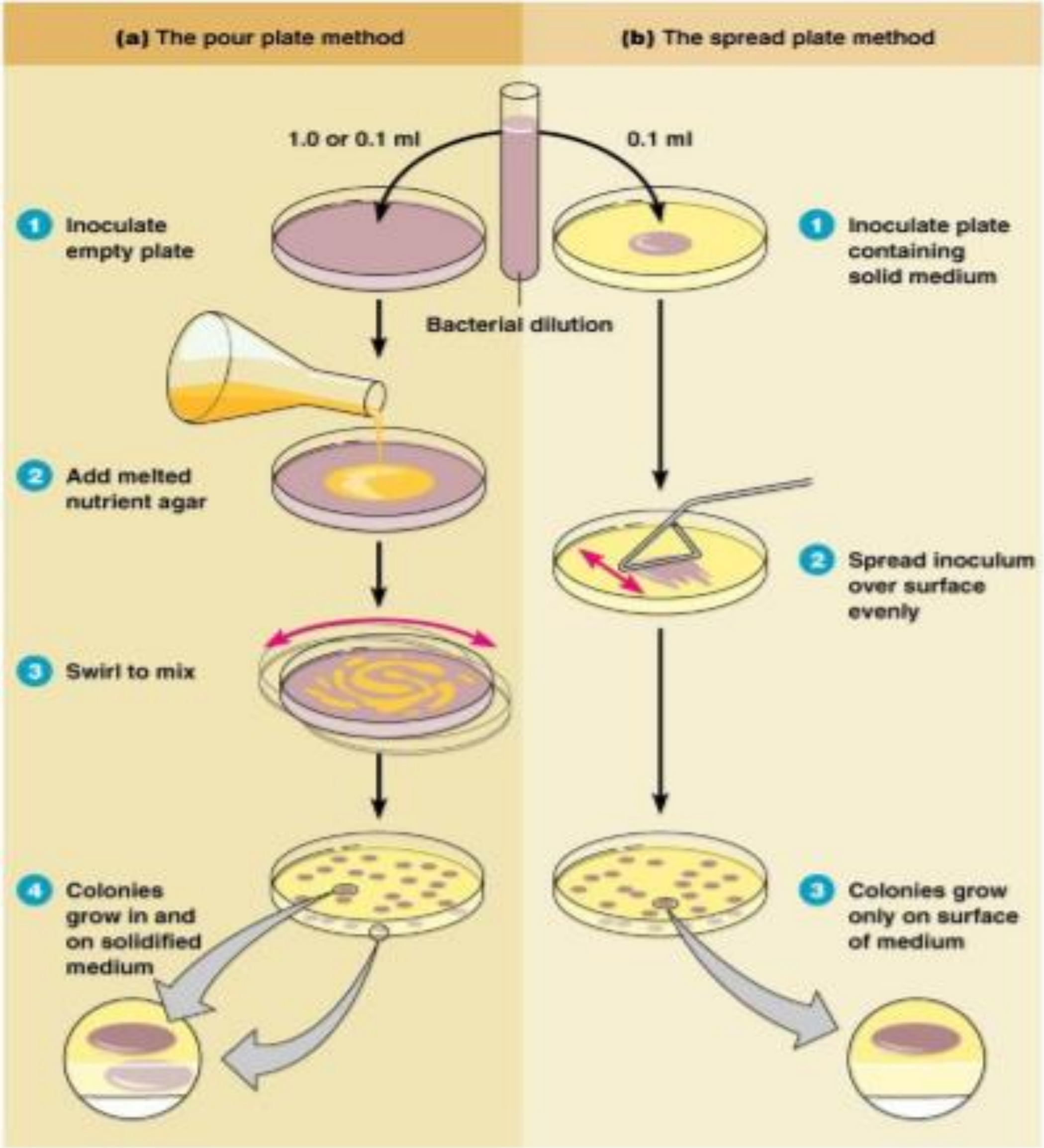


Standard Plate Count

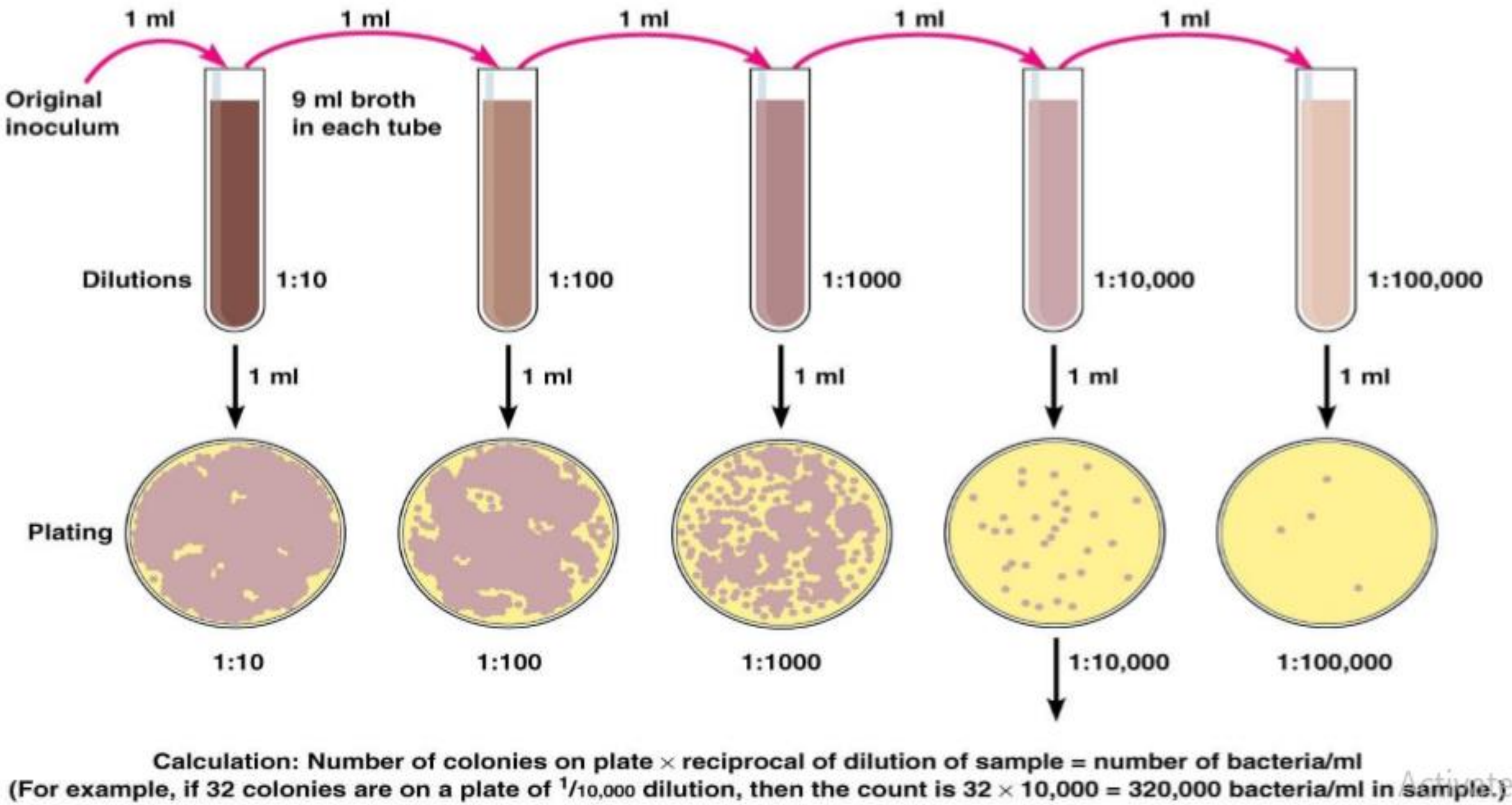
- Inoculate Petri plates from serial dilutions, There are 2 methods:

1.Pour Plate count.

2. Spread Plate count.



- After incubation, count colonies on plates that have 25-250 colonies (CFUs)



Activate
Go to Setti

Requirements for growth

1.Enviromental conditions

2.Nutrients

3.Sourse of energy.

- Important environmental factors that affect microbial growth:

1.pH

2.Temperature

3.Oxygen requirement

4.Pressure

5. Other environmental factors e.g radiation, water activities, salt concentration etc.

Factors Affecting Growth

- **Environmental Factors**

1. PH : measure of [H+].

- The acidity or alkalinity of an environment can greatly affect microbial growth.
- The control of intracellular pH is required in order to prevent the denaturation of intracellular proteins.
- Each organism has a specific requirement and pH tolerance range.
- Yeasts and moulds are typically tolerant of more acidic conditions than bacteria.
- Most micro-organisms grow best at neutral pH (7.0), but some organisms have evolved to grow best at low or high pH.

Organisms classified into 3 categories according to the optimal pH for growth:

1. Acidophiles : organisms that grow best at low pH
   (Helicobacter pylori, Thiobacillus thiooxidans)
2. Alkaliphiles : organisms that grow best at high pH
   (Vibrio cholera)
3. Most of pathogenic bacteria are neutrophiles.

Each organism has a pH range and a pH optimum.

- acidophiles – optimum in pH range 1-4
- alkalophiles – optimum in pH range 8.5-11

  lactic acid bacteria – 4-7

  Thiobacillus thiooxidans – 2.2-2.8

  Fungi – 4-6

Internal pH regulated by BUFFERS and near neutral adjusted with ion pumps.

2. Temperature

Organisms exhibit distinct growth temperatures :

1.Minimum 2.Maximum 3.Optimum

Minimum Temperature: Temperature below which growth ceases, or lowest temperature at which microbes will grow.

Optimum Temperature: Temperature at which microbial growth rate is the fastest.

Maximum Temperature: Temperature above which growth ceases, or highest temperature at which microbes will grow.

As temperature influences enzymic reactions it has an important role in promoting or preventing microbial growth.

- Four groups depending on their optimum growth temperature and the temperature range at which they will grow.

1. Hyper thermophiles have optimum growth at 100 °C and a growth range of 80-120°C .

2.Thermophiles have optimum growth at 55 °C and a growth range of 30 - 75 °C

3. Mesophiles have optimum growth at 35 °C and a growth range of 10 - 45 °C

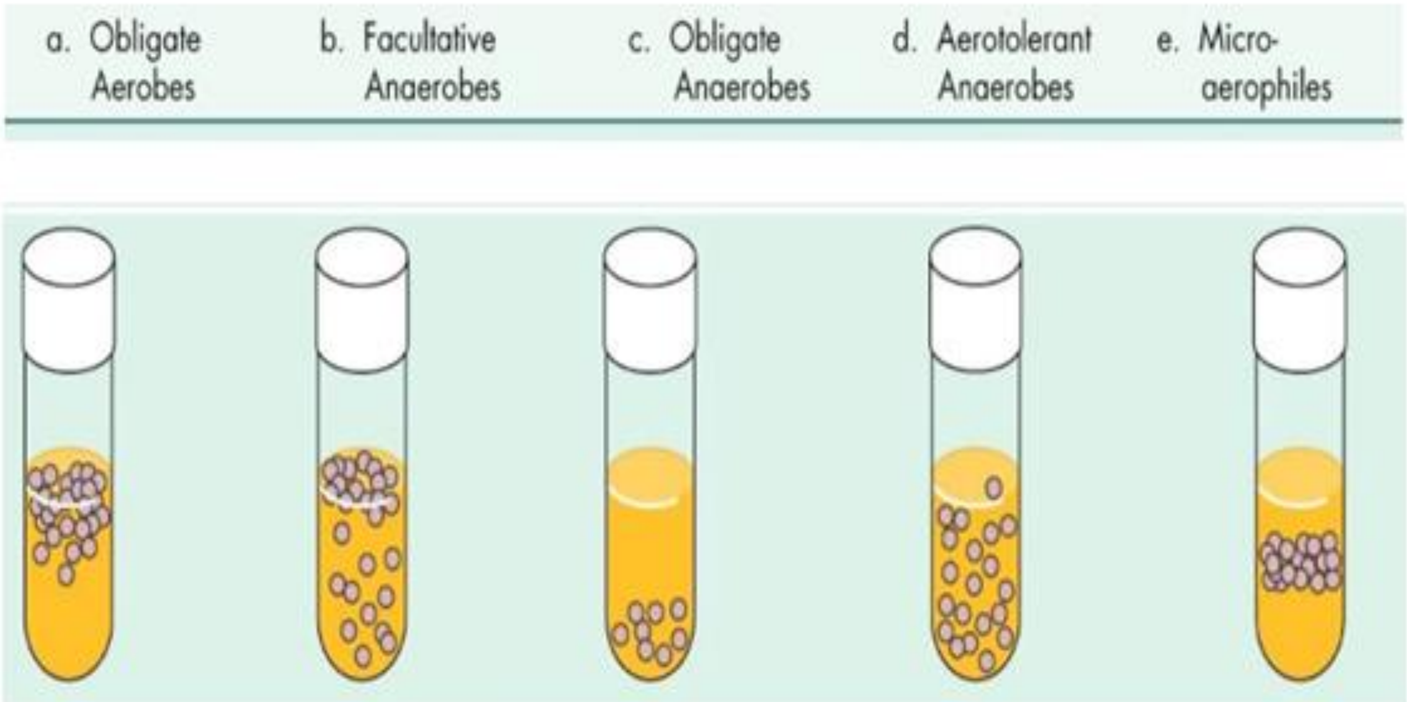
4. Psychrophiles have optimum growth at 15 °C and a growth range of -5 - 20 °C

3.Oxygen

- The atmosphere of earth contains about 20% (v/v) of oxygen. Microorganisms capable of growing in the presence of atmospheric oxygen are called **aerobes** whereas those that grow in the absence of atmospheric oxygen are called as **anaerobes**.
- The micro-organisms that are completely dependent on atmospheric oxygen for growth are called **obligate aerobes** whereas those that do not require oxygen for growth but grow well in its presence are called as **facultative anaerobes**.

3.Oxygen cont.

- **Aerotolerants** (e.g. Enterococcus faecalis) ignore O2 and can grow in its presence or absence.
- In contrast, obligate anaerobes (e.g., Bacteroids, Clostridium) do not tolerate the presence of oxygen at all and ultimately die.
- Few microorganisms (e.g., Campylobacter) require oxygen at very low level (2-10%) of concentration and are called as **microaerophiles .** And they are damaged by the normal atmospheric level of oxygen (20%).



Need Oxygen	prefer Oxygen	Oxygen Toxic	Ignore Oxygen	Reguire <2-10% O2

Nutrient Requirements

The Common Nutrient Requirements are:

1. Macro elements (macronutrients) – H, O, C, N, S, P – required in relatively large amounts

2. Micronutrients (trace elements) – K, Ca, Mg, Fe, Cu, Mn, Zn, Co, Mo and Ni – required in trace amounts – often supplied in water or in media components

3. Growth Factors

Carbon

• Classification of microorganisms on the basis of carbon source into:

1. **Photoautotrophs** – Use CO_2 as the principal carbon source .
2. **Photoorganotrophs**, Use light as energy sourse but need some energy compound – acetate as a source of carbon.
3. **Chemoautotrophs (Lithotrophs)**, Use CO_2 as the sourse of carbon & Obtain energy by the oxidation of reduced organic substances.
4. **Heterotrophs**, Use organic molecules as their source of carbon . versatile in their ability to use diverse sources of carbon.

Nitrogen

Major component of protein and nucleic acid

• Most organisms obtain N in the oxidized form of nitrate

Sources of nitrogen

• Organic molecules (amino acids)

• Ammonia (NH_3)

• Nitrate via assimilatory nitrate reduction

• Nitrogen gas via nitrogen fixation

Sulphur

Sulfur forms part of the structure of coenzymes, and found in cysteinyl and methionyl side chains of proteins.

- Sulphate is the principal source of sulphur .
- S containing amino acids are also utilized
- Microorganisms use sulphate (SO_4^{2-}) as sulfure source → end product is hydrogen sulfide (H_2S).

Other elements

- Inorganic compounds present in the environment and those released in decomposition of organic substrates are principal sources of other major nutrient elements and micronutrients.
- Phosphorus that are bound in organic compounds is releases as phosphoric acid during decomposition.

Growth Factors

- Apart from macro- and micro-nutrients, some microorganisms require additional organic compounds in very small quantities which are essential for their metabolism, These accessory compounds are growth Factor.
- Includes – Vitamins – Amino acids – Purines & pyrimidines (for synthesis of nucleic acid) – Sterols etc.
- Bases of nucleic acids
- Adenine and guanine are purines
- Cytosine, thymine, and uracil are pyrimidines