Republic of Iraq
Ministry of Higher Education
And Scientific Research
University of Baghdad
College of Science
Department of Biology

Practical Microbial physiology

2020 – 2021

المرحلة الثالثة ـالدراستين الصباحية والمسائية

الفصل الدراسي الاول

تدريسيو المادة

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Lab -1- *Culture Media*

Growth medium or culture medium *is combination of substances designed to support the growth of microorganisms or cells, Different types of media are used for growing different types of cells.*

Pure culture *:culture medium containing the growth of single species of bacteria and we can preserve it by*
1-Cooling ***2-Freezing*** ***3-Lyophilization(Freeze drying)***

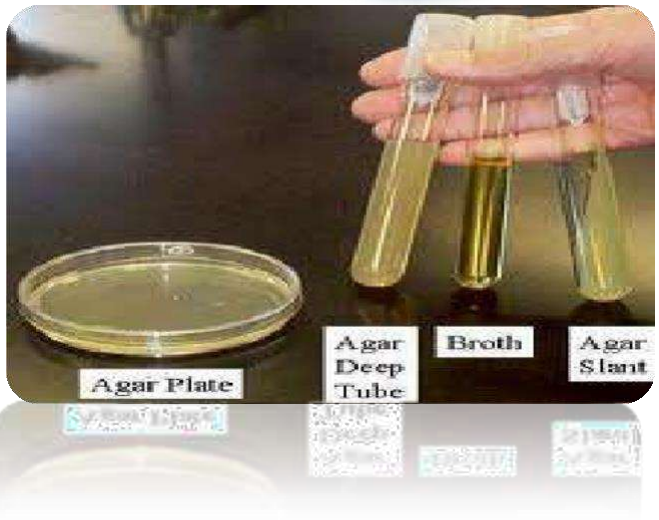
Mixed culture : *culture medium containing the growth of two or more species of bacteria*

Kinds of culture media

Culture media can be divided according to
1-Their consistency
a-Solid media ***2%*** *agar*

b-Semisolid media ***1%*** *agar*

c-Liquid media ***0%*** *agar*



Agar

Is a complex carbohydrate extracted from sea algae called Gelidium ,used in preparing culture media as solidifying agent because of its characteristics which are :
1-Its ***melting*** *properties,* ***melt at 90-100C˚*** *and* ***solidify at 42C˚.***
2-It has ***no nutritive*** *value for majority of bacteria.*

2-According to use and contents

Natural media(non-synthetic)

Media contain natural material rich with vitamins and their structure and concentration are not defined such milk and blood

Defined media (synthetic media)

Medium contain chemical materials their structure and concentration exactly defined

Semi –synthetic media

Media contain natural material as well as chemical materials

Living media ***medium contain living tissue used to culturing viruses and cancer cell***

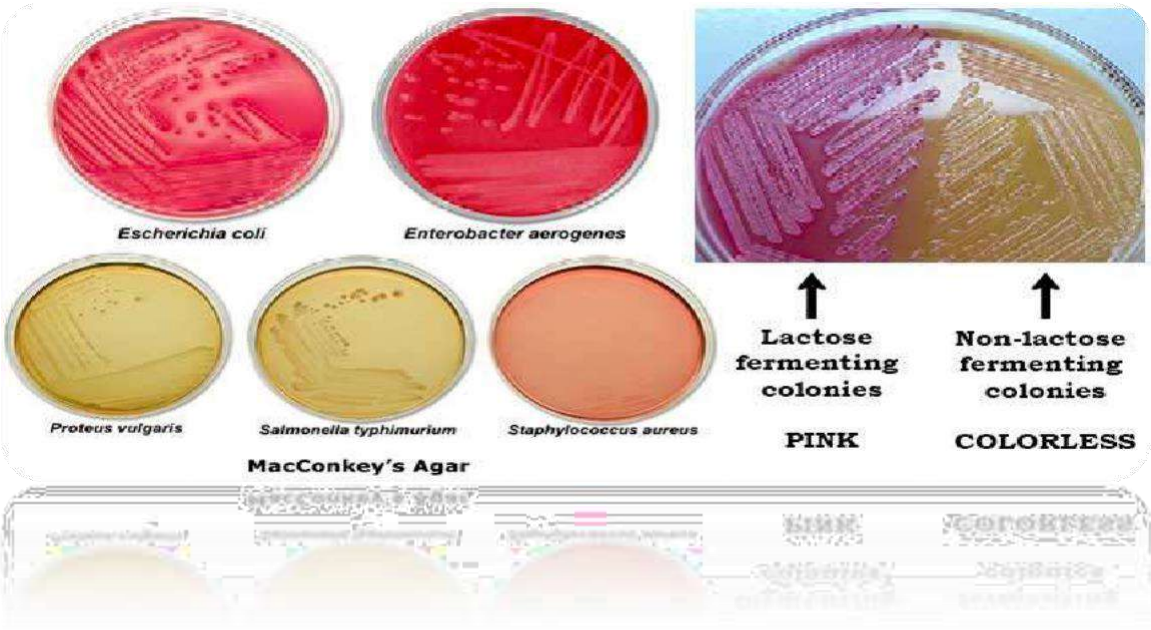
Routine Laboratory Media

1. Basal media. ***Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.***

2. Enriched media ***The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.***

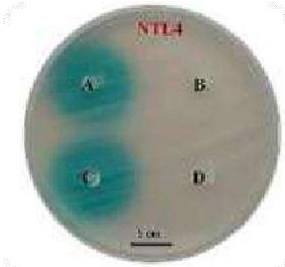
3. Selective media. ***These media favor the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: EMB***

4. Differential media (Indicator). ***An indicator is included in the medium. A particular organism causes change in the indicator, e.g. MacConkey agar are differential media(contain lactose sugar and neutral red).***



5. Transport media. ***These media are used when cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.***

6. Preservation media. ***Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth.***



7-Assay medium ***Medium used to assay the production amount of some material in bacteria***

8-Enumeration media ***that used to calculate the number of bacteria in water ,soil and food sample***

9-Characterization media ***that used to characterize and recognize type of bacteria***

Preparation of culture media

1-Weightining ***the medium ingredients according to the direction written on its container.***

2-Dissolve ***with little amount of D.W. then complete the volume to the volume you want and may be need using heating and stirrer for complete dissolving.***

3-Check pH .

4-Dispensing ***the medium in to test tube by pipette.***

5-Sterilization ***by autoclave.***

6-Dispensed ***agar medium into petri dish when the heat reach to 45.***

EX :prepare 500ml of N.A. medium if the direction on container wrote 8gm/liter

gm	*ml*
8	*1000*
x	*500*

x=8 * 500/1000 = 4 gm of media dissolve in little amount of D.W. then complete the volume to 500 ml then autoclaved and poured in plates

Method of pouring the media in plate

The sterile plates should be on the table near the burner then
Cooling ***the solid medium to 45C˚ to avoid solidify it and to avoid forming of drop on the cover of plates***
Remove ***the cover (or cotton plug) and sterile the upper part by burner***
Remove ***the cover of plate near the burner and pouring the medium and close the cover of plate***
Moving ***the plate on table 5 times in two direction to distribute the media equally in plate.***

Sterility test

This test mean putting the flasks tubes and plates which contain sterile media before using in incubator at 37C for 24 hr. to ensure that there is no contamination while preparing and pouring the media

Lab -2- Bacterial Counting

In order to observe microbial reproduction, it is necessary to determine numbers or mass of M.O. the estimation of microbial population or count could be:

Total count (counting living and non-living cells)

Cell growth is cane bmeasured by counting total cell number of the microbes present in that sample. Total cells (both live and dead) of liquid sample are counted by different methods.

1-Breed method
2-Haemocytometer (counting chamber)
3-Optical density (O.D.) by spectrophotometer
4-Dry weight method
5-Wet weight method
6- Coulter Counter

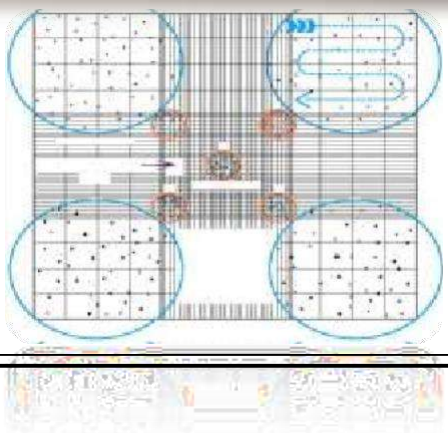
Total count methods

1-Breed method

1-Draw a square (1 cm^2) by wax pen on the slide .
2-Put 0.01 ml (one loop full)from broth culture (Bacillus or Staph aureus)on the back of the square.
3-Make a smear inside the lines of the square.
4-Fix on the flame
5-Wash with tap water.
7-Examine under oil-immersion objective lenses.
8-Count the cells in 10 fields. Then find the range of this count and use the following formula:-

No. of cell/ 1 ml= the average no .of cells in 10 field x 5000 x 100 x inverse of dilution if used

2-Haemocytometer (counting chamber)



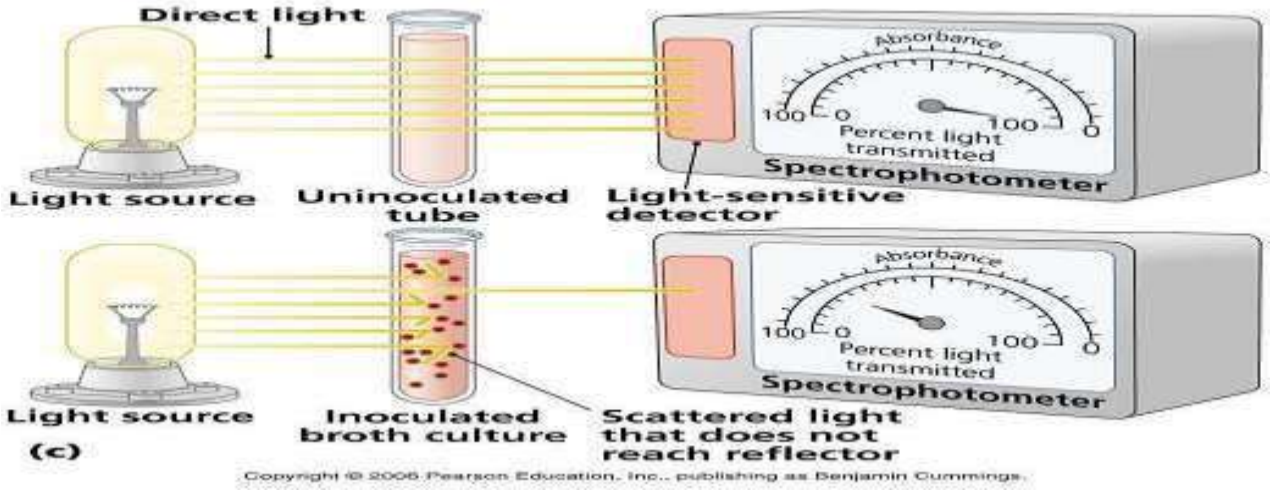
1-Put the cover on the counting chamber
2-Put 0.01 ml (one loop full) of culture near the edge of the cover , the drop will spread (under the cover)
3-Count the cells as the following.
Count the cells in squares (4 corners and one in the middle)if the chamber with 25 squares , used the following formula :
No. of cells/1 ml=no. of cell in 5 squares x 5 x 10 x 1000 x inverse of dilution if used
Count the cells in 4 squares (4 corners only) if the chamber with 16 squares , and used the formula:
No. of cells/1 ml=no. of cell in 4 squares x 4 x 10 x 1000 x inverse of dilution if used

3- Absorbance:

Absorbance is measured by using a spectrophotometer. Scattering of light increases with increase in cell number. When light is passed through bacterial cell suspension, light is scattered by the cells.

Therefore, transmission of light declines. At a particular wavelength absorbance of light is proportional to the cell concentration of bacteria present in the suspension.

Thus cell growth of any bacterial suspension at a particular wavelength at different intervals can be measured in terms of absorbance and a standard graph (between absorbance and cell concentration) can be prepared.



4- Dry Weight Measurement:

Used for fungi in many times.

Dry weight measurement of cell material is similar to that of wet weight. Here dry weight of pre-weighed filter paper containing pellets of microbial cells is measured. Dry weight of filter paper is nullified by subtracting the dry weight of only filter paper of similar size.

Thus dry weight of microbial cells can be obtained. For example dry weight of about one million cells of E. coli is equal to 150 mg. Dry weight of bacterial cells is usually 10-20% of then- wet weight.

5- Wet Weight Measurement:

Measuring cell mass is an easy step of cell growth measurement. A known volume of culture sample from the ferment or is withdrawn and centrifuged, Wet weight of pellets is measured by using pre-weighed filter paper. A pre-weighed filter paper of similar size is used to subtract the weight of wet filter paper. Thus wet-weight of cells is calculated.

6- Coulter Counter

It is an electronic device. The microbial culture is directly used to count cells present in the suspension.

Viable count

A viable cell is defined as a cell which is able to divide and increase cell numbers. The normal way to perform a viable count is to determine the number of cells in the sample which is capable of forming colonies on a suitable medium.

Here it is assumed that each viable cell will form one colony. Therefore, viable count is often called plate count or colony count. There are two ways of forming plate count.

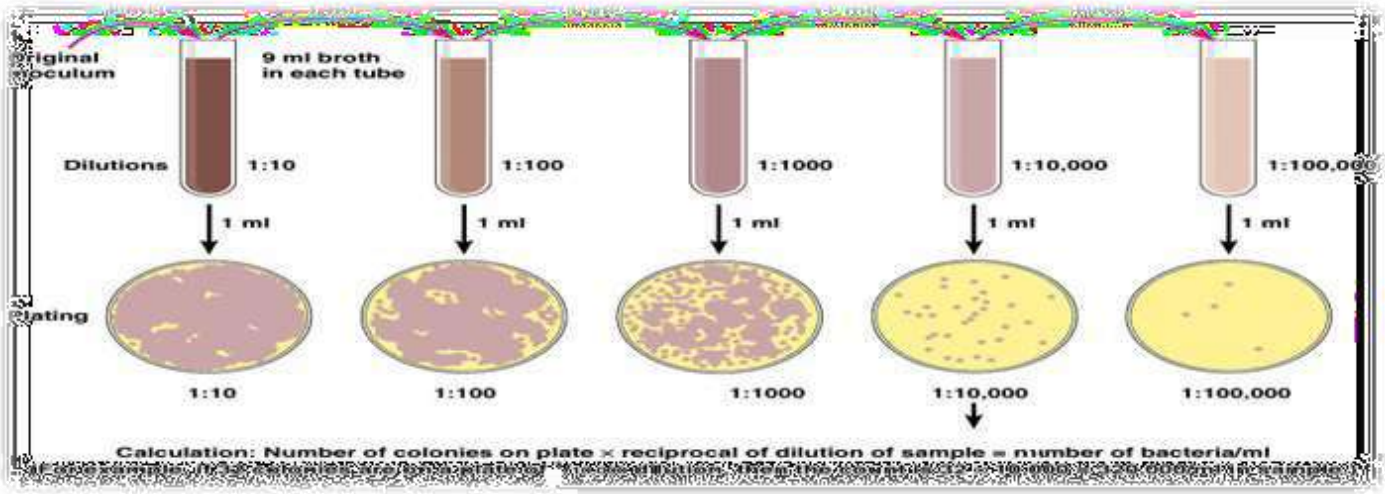
1-Dilution to extinction
2- Most probable number (MPN)
3- Pour plate method
4-Spread plate method
5-Membrane filter method

1- Dilution to extinction

In this methods , serial dilutions of sample containing viable M.O. are plated onto suitable growth medium. This method is used to determine the viable population in sample containing bacteria.

After the culturing , the plates are then incubated under conditions that permit microbial reproduction. It is assumed that (each bacterial colony arises from individual cell that has undergone cell division)

Thus , by counting the no. of colonies and counting for the original dilution factor, the number of bacteria in the original sample can be calculated , which is a way to obtain pure culture , but the major disadvantage of this method that is selective because there is no universal set of conditions that permits the growth of all M.O.



Dilution are achieved by adding an aliquot of the specimen to sterile water tube

1-if 1 ml of sample is added to 9 ml of sterile water,the dilution is 10^{-1} or adding 0.1 ml of sample to 9.9 ml sterile water , the dilution is 10^{-2}

2-greater dilutions are achieved by sequentially diluting the sample in series.

Or

Adding 0.1 ml from the first dilution(10^{-2}) to 9.9 ml of sterile water , so the total dilution is 1:10000= 10^{-4}

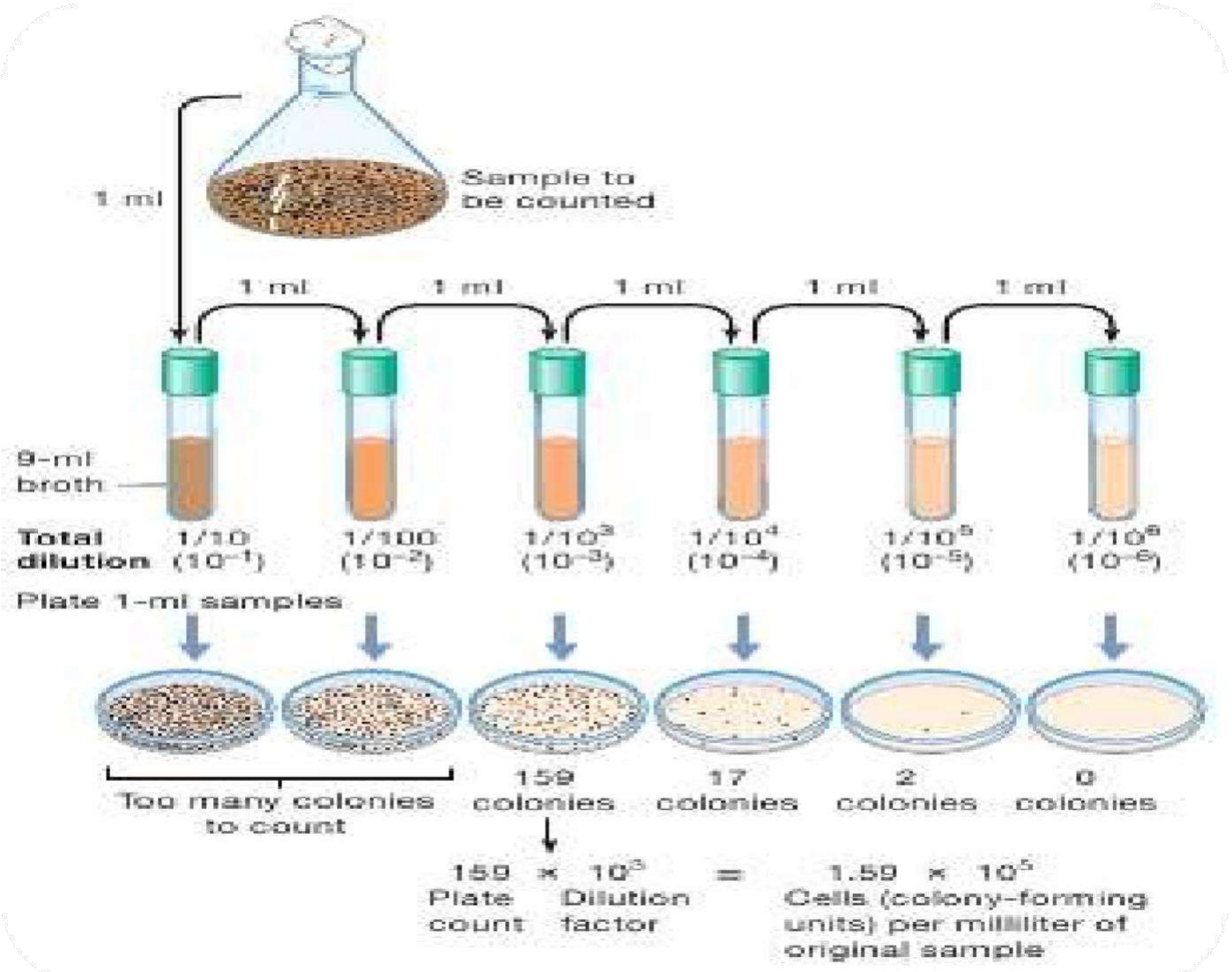
The formula use in dilution is:

Dilution = Part(the transfer volume)/ All(transfer volume+ diluents volume)

The types of liquids are used as diluents

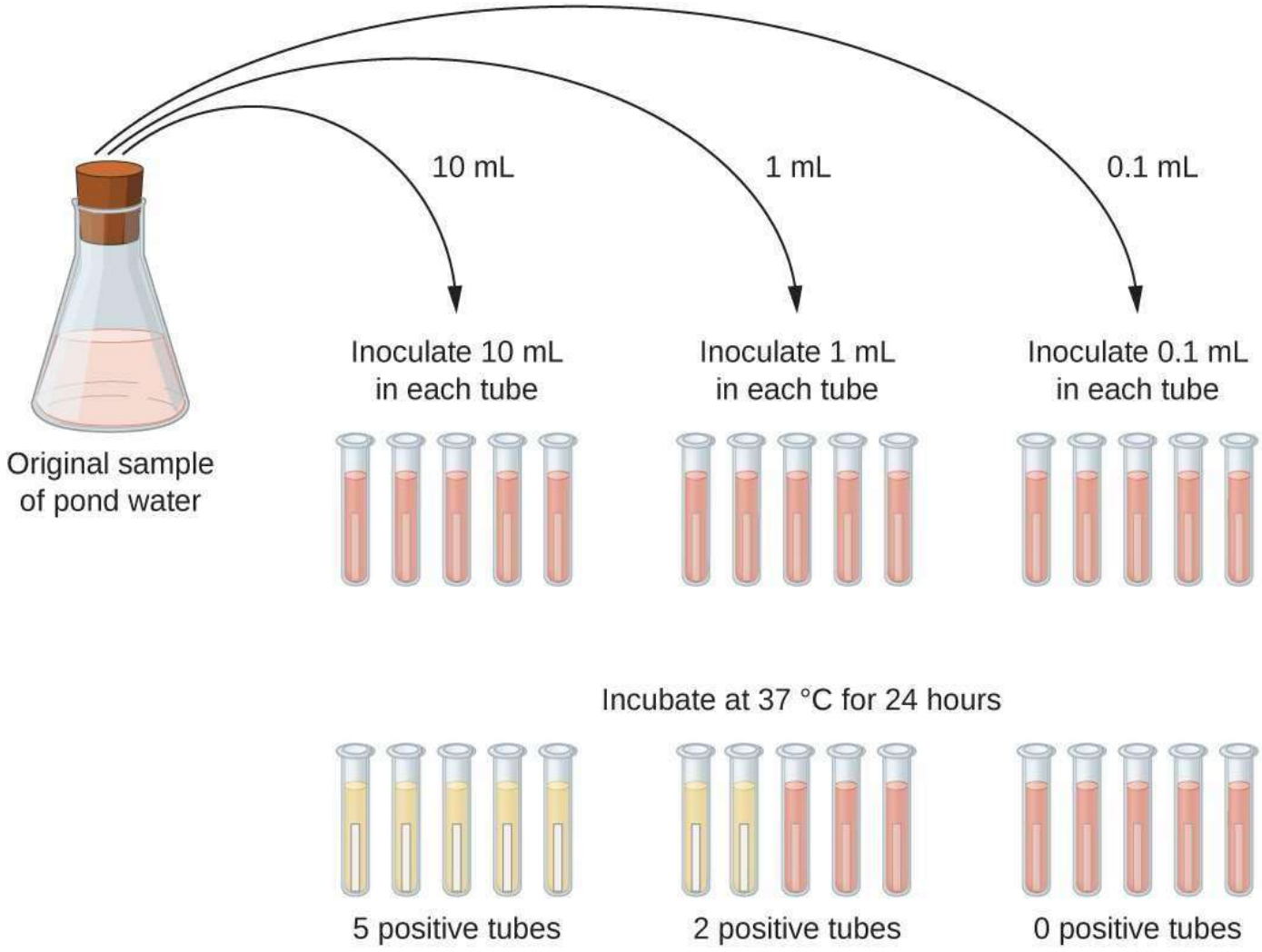
1-Saline(NaCl=0.85%) ***2-Sterile distilled water***

3-Peptone water (0.1%) ***4-Nutrient broth (N.B.)***



2- Most probable number (MPN)

Most Probable Number (MPN) is a method used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions. It is commonly used in estimating microbial populations in soils, waters, agricultural products and is particularly useful with samples that contain particulate material that interferes with plate count enumeration methods.

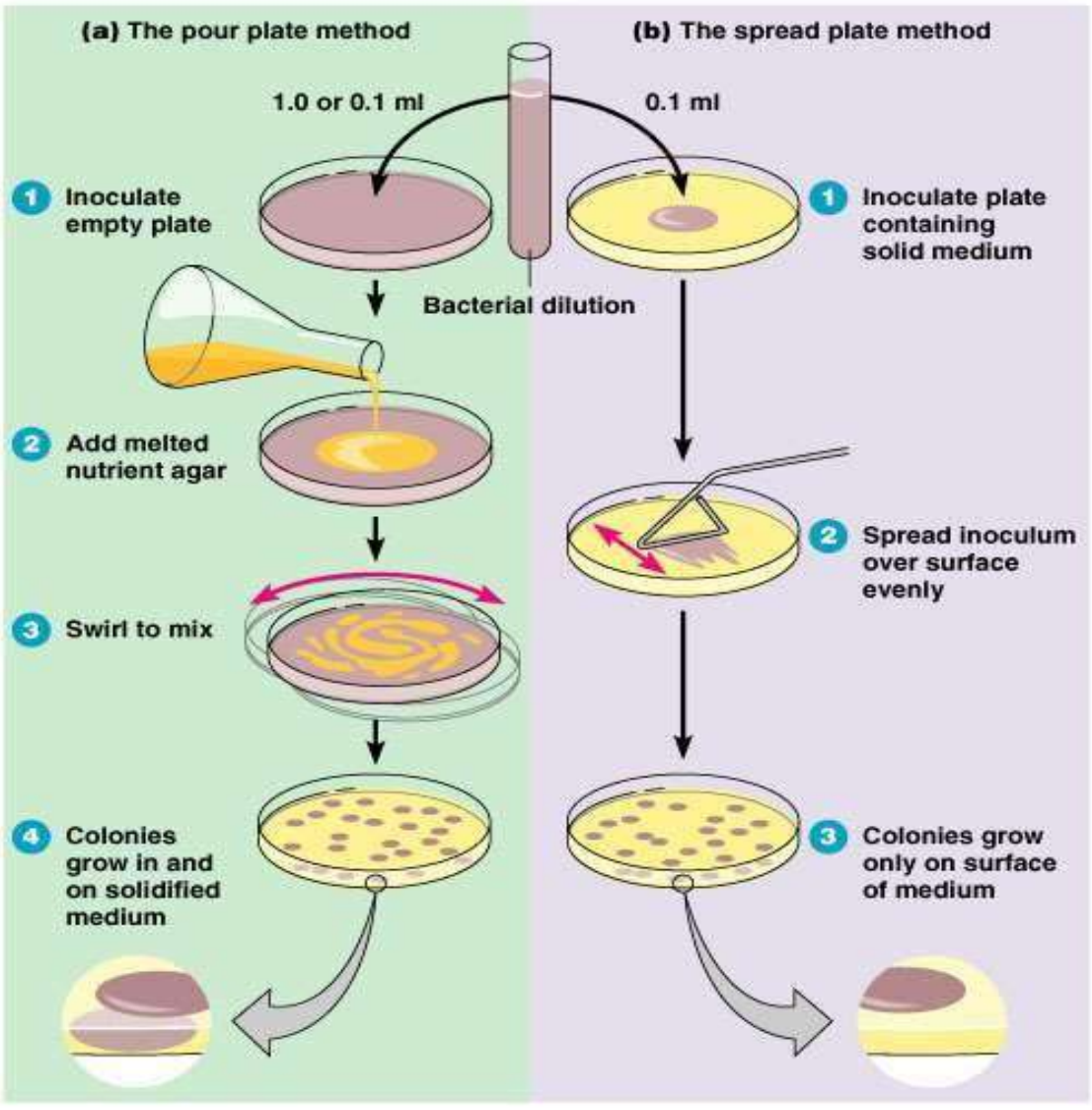


3- Pour plate method

In this method a known volume (0.1-1.0 ml) of the culture is poured into the sterile Petri dishes. Then melted agar medium is poured and mixed gently. The plate is incubated. Colonies growing on the surface of agar are counted.

4-Spread plate method

A volume of culture (0.1 ml) is spread over the surface of an agar plate by using a sterile glass spreader. The plate is incubated to develop colonies. Then the number of colonies is counted



Lab -3- Bacterial Growth Curve

Bacterial growth is the 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. Hence, "local doubling" of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists;

the basic means requires

bacterial enumeration (cell counting) by direct and individual (microscopic),
direct and bulk (biomass),
indirect and individual (colony counting),
indirect and bulk (most probable number, turbidity, nutrient uptake) methods.

Batch culture

Bacterial growth curve\Kinetic Curve

The growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases:

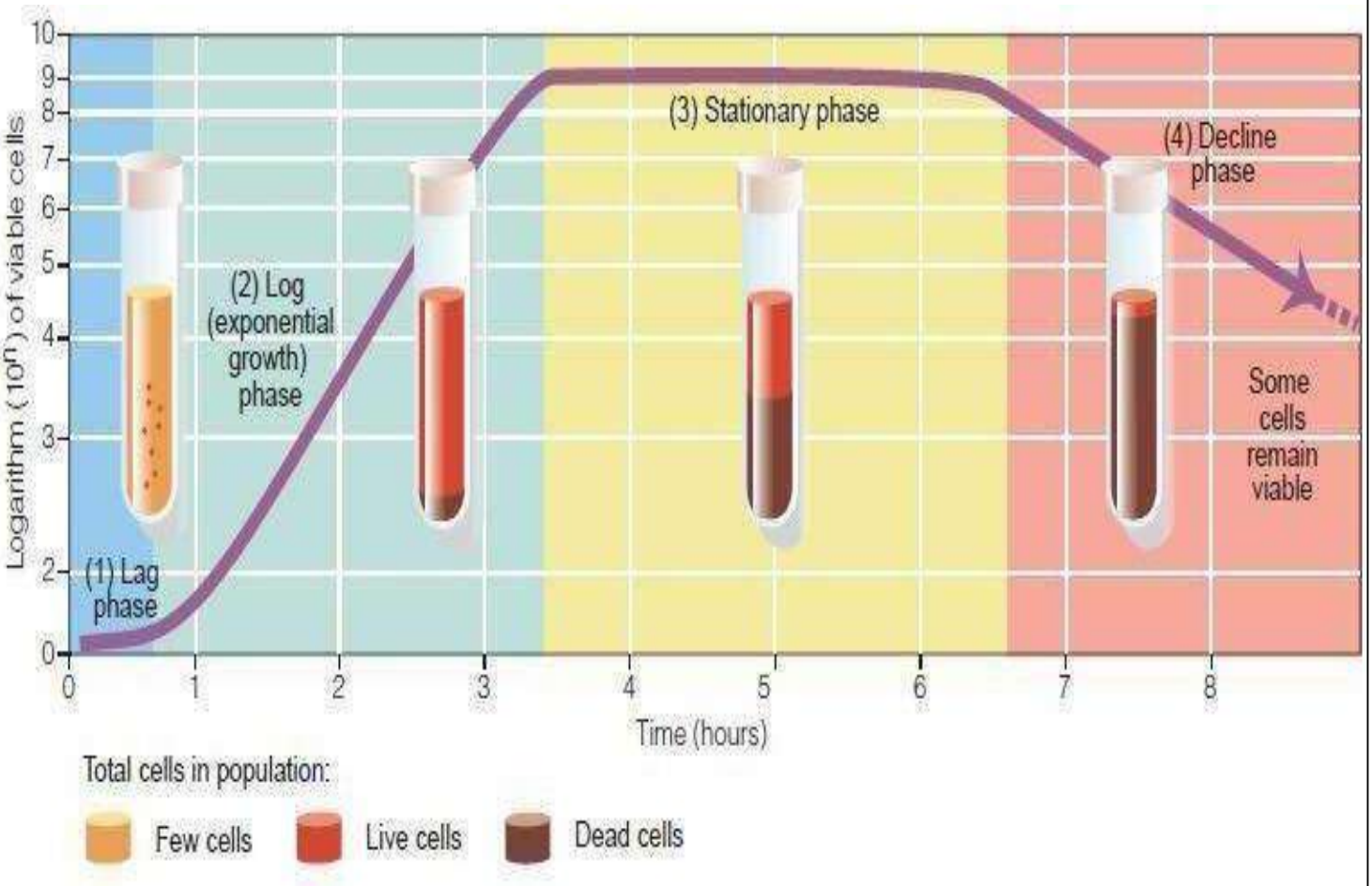
1- lag phase (A),
2- log phase or exponential phase (B),
3- stationary phase (C),
4- death phase(D).

During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.

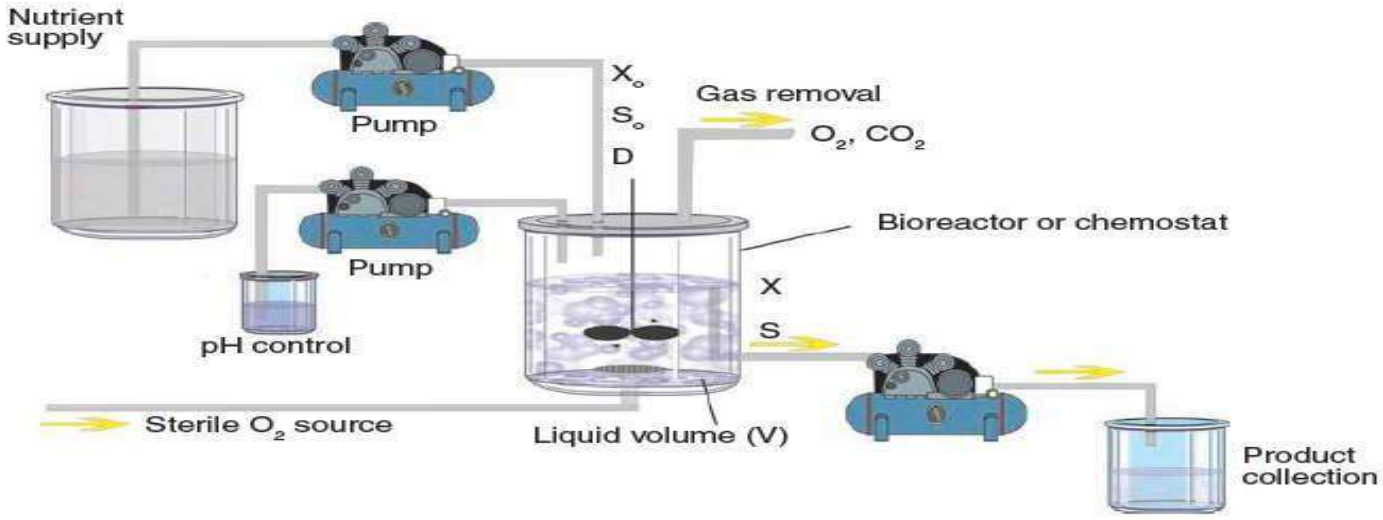
The log phase (sometimes called the logarithmic phase or the exponential phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can double their population four times a day. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

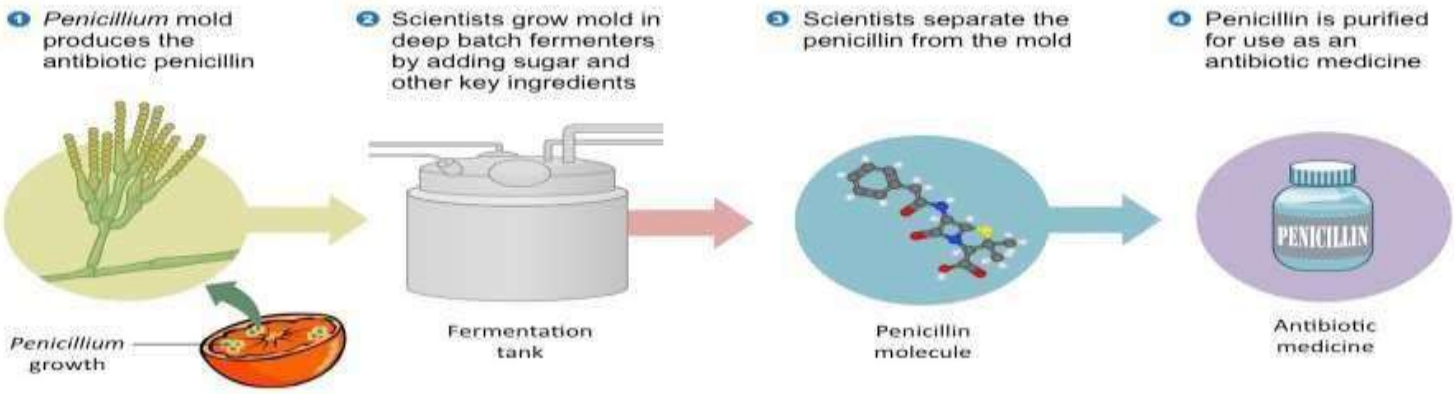
The stationary phase ***is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a –smooth,‖ horizontal linear part of the curve during the stationary phase. Mutations can occur during stationary phase. Presented evidence that DNA damage is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria. Endogenously generated reactive oxygen species appear to be a major source of such damages.***

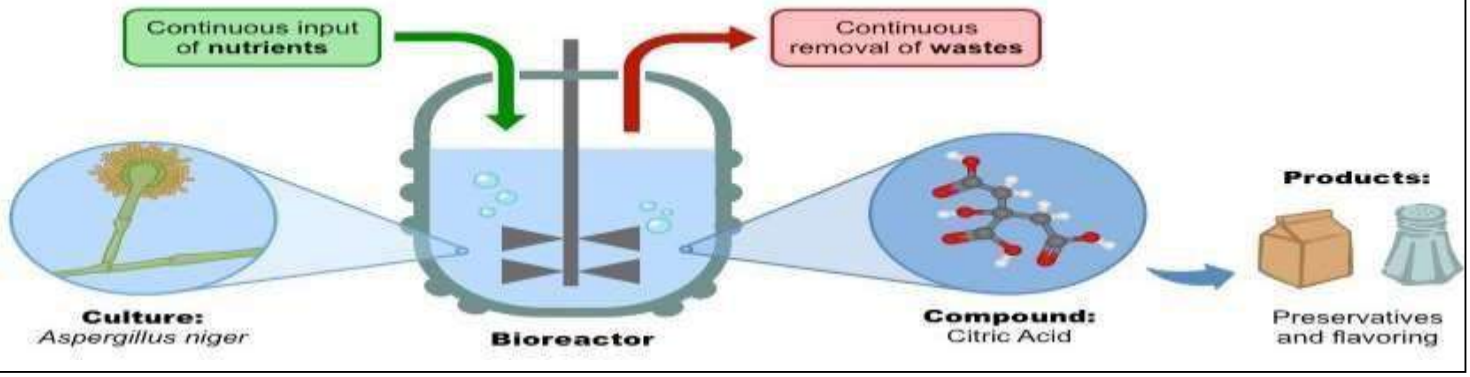
At death phase ***(decline phase)*** ***bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.***



Continuous culture ***of micro-organisms is a technique of increasing importance in microbiology. The essential feature of this technique is that microbial growth in a continuous culture takes place under steady-state conditions; that is, growth occurs at a constant rate and in a constant environment. Such factors as pH value, concentrations of nutrients, metabolic products and oxygen, which inevitably change during the _ growth cycle ' of a batch culture, are all maintained constant in a continuous culture; moreover, they may be independently controlled by the experimenter.***







$$n = \log b - \log a / 0.301$$

n= number of doubling or number of generation

b= the number of cell at present or given time

a=number of cell at original inoculums

$$gt = T2 - T1 / n$$

gt= generation time (min)

T2 time when no. of cell =b

T1 time when no. of cell =a

$$K = 2.303 (\log b - \log a) / T2 - T1$$

K=growth constant

$$K = gt / 0.693$$

Or

$$gt = K / 0.693$$

Lab -4- Growth Yeild

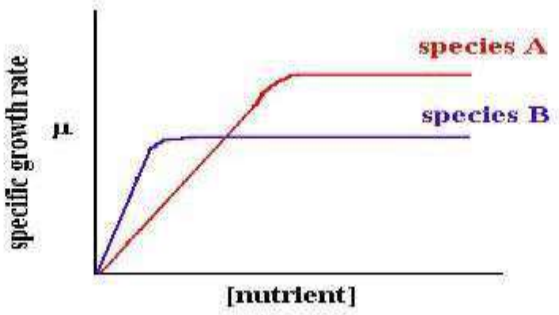
There are more than one expressions in use for bacterial growth yield. The simplest term is biomass yield (Y) described as biomass produced per unit of substrate (limiting nutrient)consumed. Biomass produced can be determined by different methods. The amount of substrate consumed is the difference between the initial concentration and the concentration of substrate left after the growth period.

Limiting nutrient: the nutrient(glucose mostly) that the organism cannot grow without it. and it produce a constant biomass per each unit of it.

In order to get fast and regular of chemoheterotrophic M.O. population growth , its necessary to use enrichment media which supply limiting nutrients in their reduced state. That cultivate M.O. readily incorporate these nutrient in cell compound without losing cellular energy in reducing activities ,this enhance growth rate ,but in cultivation in minimal media which lack certain growth factors regular increase not obtained

Growth yield standard curve used in bioassay techniques by using certain growth nutrients. In this technique standard bacterial strains used in estimation of trace elements in food sample or others. In isolation of vitamins like B12(biotin), and used in clinical diagnostic practice in case of many human syndromes such as in diagnosis of phenyl ketoneuria (mental retardation in human) as a result of enzyme deficiency which convert phenyl alanine to tyrosine that results of phenyl alanine accumulation in patient serum, this syndrome is still diagnosed until this time by cultivation of bacteria require this amino acid in cultural medium luck this amino acid and mixed with patient serum , positive result indicate by test bacteria growth , then concentration of phenyl alanine amino acid determined by bioassay.

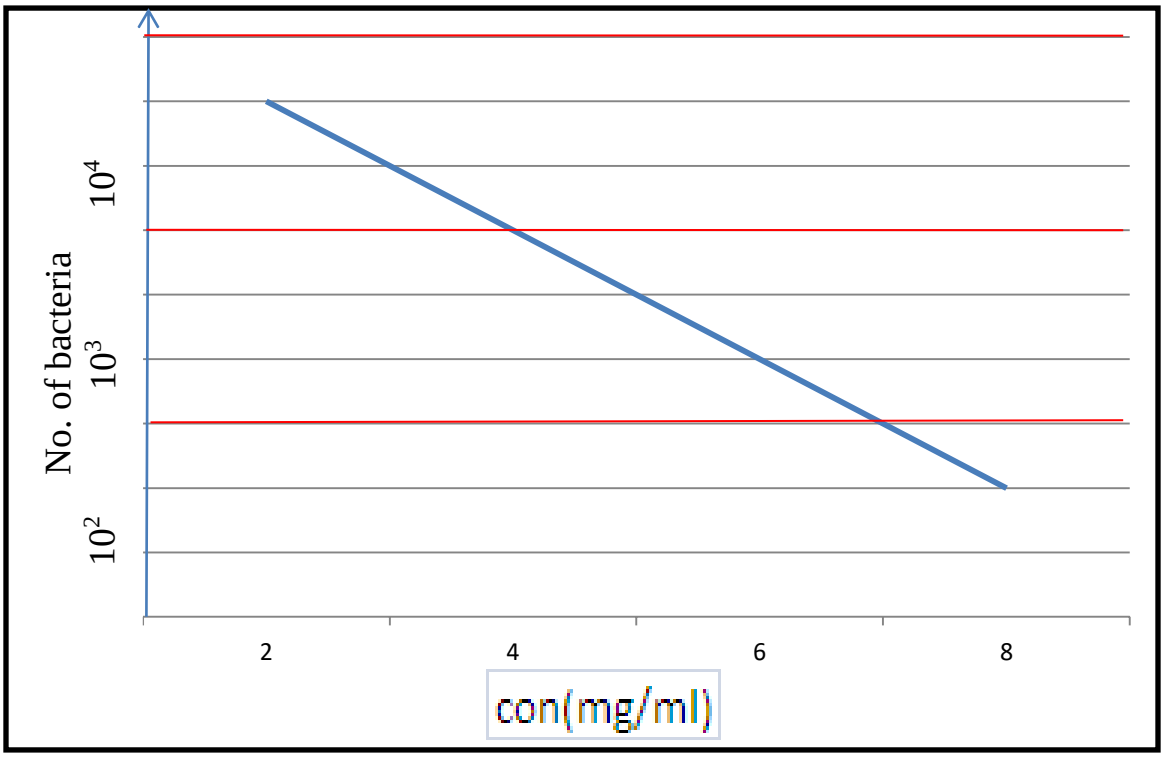
The growth yield can be mathematically expressed as follows:



$$Y = b - a / C_o - C$$

$$Y = \log b - \log a / C_o - C$$

b: is the dry wight or no. of cell /ml at point of growth just before the stationary phase
a; is the dry wight or no. of cell /ml just after inoculation
C_o: is the initial concentration of limiting nutrient
C: is the concentration of limiting nutrient when growth is stop



Bacterial efficiency

M.O. are not 100% efficient in incorporating cultural nutrients like carbon and energy to cellular compounds even in lab.

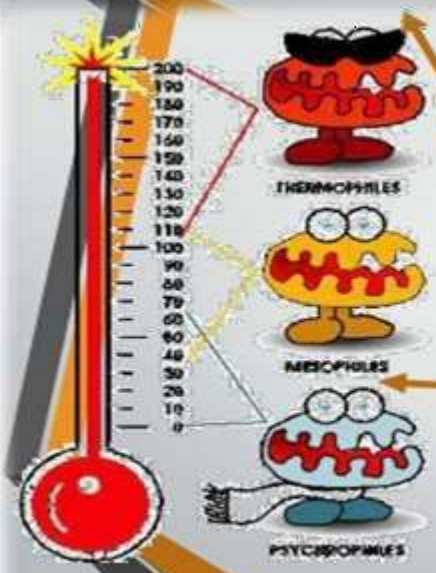
Optimal condition researchers revealed that nutrient conversion efficiency ranging 20-25% and thus explained in relation to high energy compounds production in case of cultivation bacteria in enrichment media, most of organic nutrients consumed in energy production and in active transport and little inter to cell structures.

Lab -5- Microbial growth requirements

Chemical Requirements

- **Carbon**
  - Carbon is the structural backbone of all living matter
  - Organic compounds
- **Nitrogen, Sulfur, Phosphorus**
  - N_2 and P required for synthesis of DNA and ATP
  - N_2 required for protein synthesis
- **Trace Elements**
  - Needed for enzymatic functions
  - Can be added to media to culture microbes
  - Fe, Cu, Zn

Physical Requirements



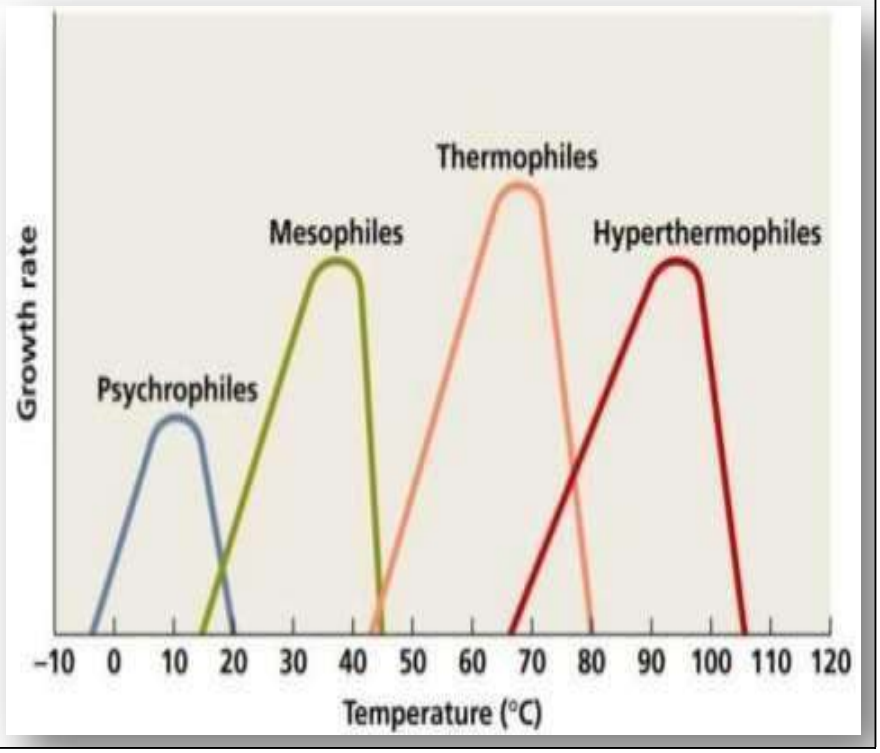
- **Temperature**
  - **Hyperthermophiles** –some members of Archaea, "extreme thermophiles"
  - **Psychrophile**-cold loving microbes
  - **Mesophile**-moderate temperatures (most common)
  - **Thermophile**-heat loving microbes
  - **Psychrotrophs**-capable of growing between 0 and 30 degrees Celsius (refrigerator temperatures)

$$Q_{10} = K_{(t+10)} / K_{(t)}$$

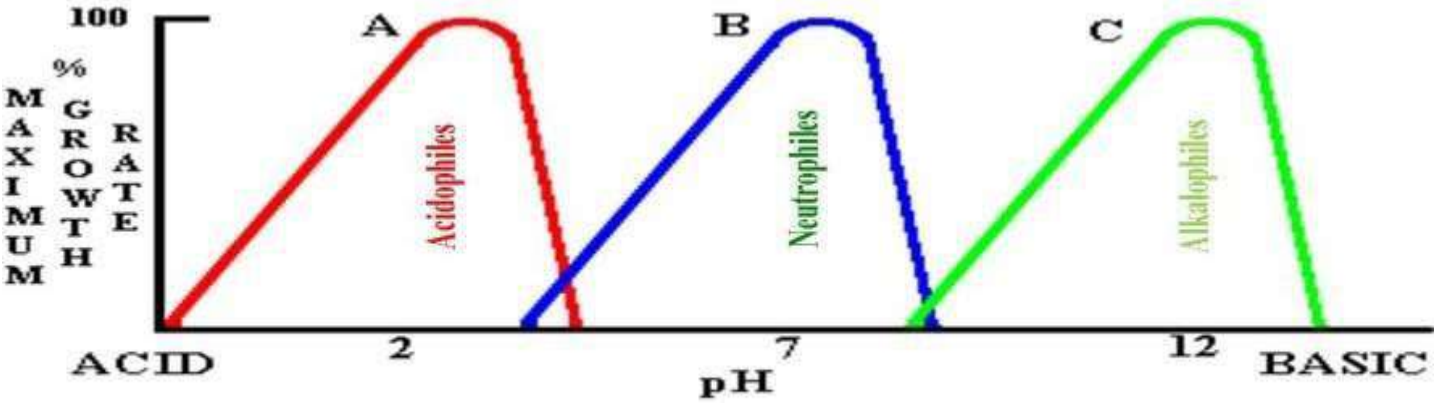
Q_{10} = 1 no effect on growth

Q_{10} < 1 growth decrease with temperature increasing

Q_{10} > 1 growth increase with temperature increasing

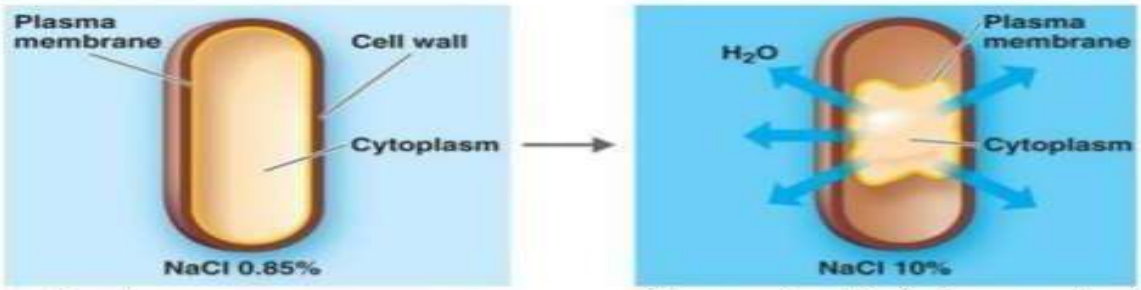


- **pH** – the acidity or basicity of a solution
  - Most bacteria growth best at a pH between 6.5-7.5
    - Neutral
- **Osmotic pressure**
  - Microbes are 80-90% water
  - Adding solutes to solutions can reduce the presence of microbes (i.e. salt)



- Most bacteria grow between pH 6.5 and 7.5
- Molds and yeasts grow between pH 5 and 6

Osmotic pressure is the pressure exerted on bacterial cells by their environment



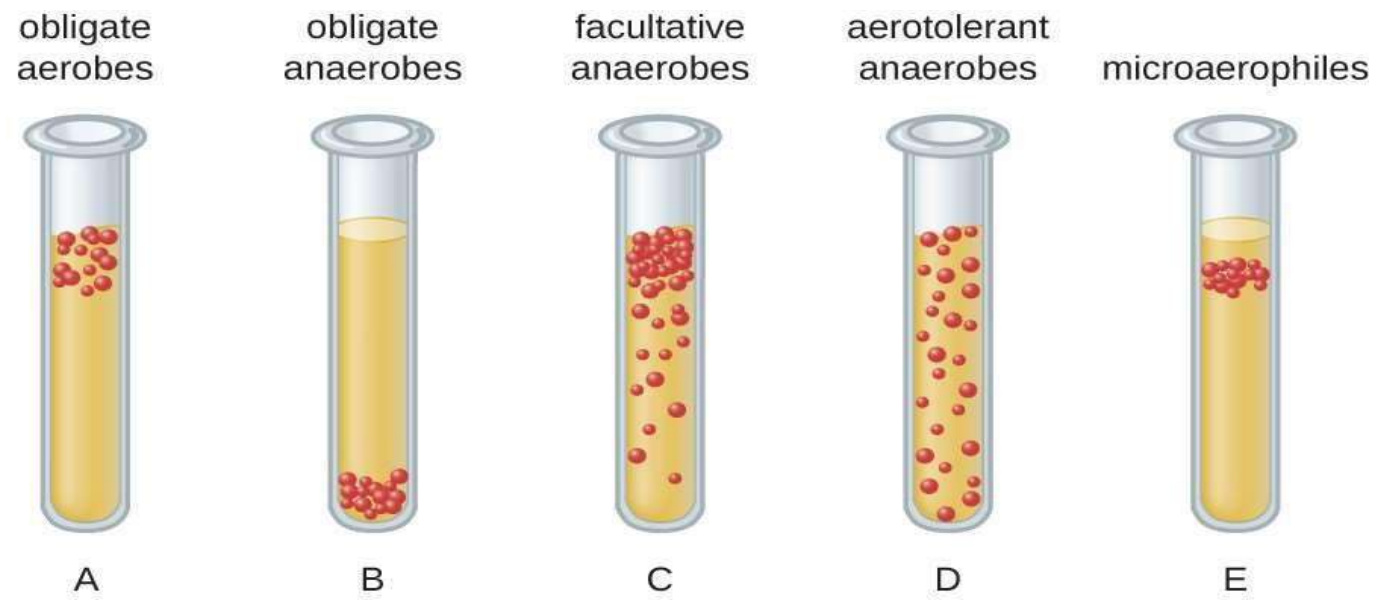
- **Isotonic**
- **Hypertonic (plasmolysis)**

Hypotonic: the bacterial cell gains water and swells to the limit of its cell wall

Some opportunistic pathogens are **facultative halophiles**

- ***Staphylococcus aureus*** **- colonizes the surface of the skin (salt)**

- **Oxygen**
  - **Obligate Aerobes-** require O2 to live
  - **Facultative Anaerobes** – can use O2 to live if present, but doesn't require it for growth
  - **Obligate Anaerobes** – do NOT require O2 to live, harmed by it
  - **Aerotolerant Aerobes-** can not use O2 for growth, but they can handle it. Not necessarily harmed by the presence of O2



Lab -6- Factor affecting on microbial growth

Irradiation

Irradiation is the use of ionising gamma rays , or, high-energy electrons and X-rays to inactivate microbial pathogens, particularly in the food industry.

Metal ions (Oligodynamic effect)

Ions of various metals have a toxic effect on microbial life by denaturing microbial enzymes and thus disrupting their metabolism. This effect is negligible in viruses since they are not metabolically active.

Pulsed electric fields (PEF)

Strong electric field pulses applied to cells cause their membranes to develop pores (electroporation), increasing membrane permeability with a consequent and, for the cell, undesirable migration of chemicals.

Pulsed magnetic fields (PMF)

A 2004 study found that E. coli is susceptible to pulsed magnetic fields. Enzymes such as lactoperoxidase, lipase and catalase are readily inactivated.

High power ultrasound

Ultrasound generates cavitation bubbles within a liquid or slurry by causing the liquid molecules to vibrate.

Low temperatures

Freezing temperatures curb the spoiling effect of microorganisms in food, but can also preserve some pathogens unharmed for long periods of time.

High temperatures

destroy viruses and vegetative cells that are active and metabolising. Organic molecules such as proteins, carbohydrates, lipid and nucleic acids, as well as cell walls and membranes, all of which play important roles in cell metabolism, are damaged by excessive heat.

High osmotic gradients

Syrup, honey, brine, alcohol and concentrated sugar or salt solutions display an antibacterial action due to osmotic pressure.

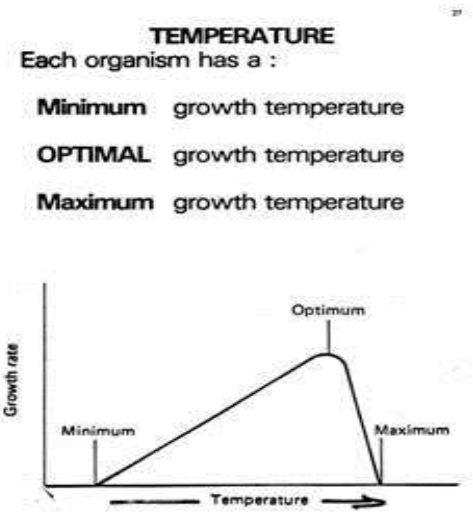
Highpressures

Water under very high hydrostatic pressure inactivates pathogens such as Listeria, E. coli and Salmonella. High pressure processing (HPP) is preferred over heat treatment in the food industry as it eliminates changes in the quality of foods due to thermal degradation, resulting in fresher taste, texture, appearance and nutrition.

High acceleration

Bacterial cell surfaces may be damaged by the acceleration forces attained in centrifuges. Laboratory centrifuges routinely achieve 5000–15000g, a procedure which often kills a considerable portion of microbes, especially if they are in their exponential growth phase.

Among all factors that affect microbial growth, temperature is probably one of the most important factors directly affecting the growth of microorganisms .Un microbial growth generally exhibits 3 characteristic temperatures—the Minimum growth temperature (Tmin), Optimum growth temperature (Topt), and Maximum temperature (Tmax). Below Tmin or above Tmax, microorganisms do not grow or may even die off. Between Tmin and Topt, or the suboptimal temperature range, the microbial growth rate increases with temperatures. Between Tmax and Topt, the microbial growth rate decreases with temperatures.



D-value

In microbiology, D-value refers to decimal reduction time (or decimal reduction dose) and is the time (or dose) required at a given condition (e.g. temperature), or set of conditions, to kill 90% (or 1 log) of the exposed microorganisms; however, it has analogous uses in other microbial resistance and death rate applications, such as those of ethylene oxide and radiation processing.

Thus after a colony is reduced by 1 D, only 10% of the original organisms remain, i.e., the population number has been reduced by one decimal place in the counting scheme. Generally, each lot of a sterilization-resistant organism is given a unique D-value.

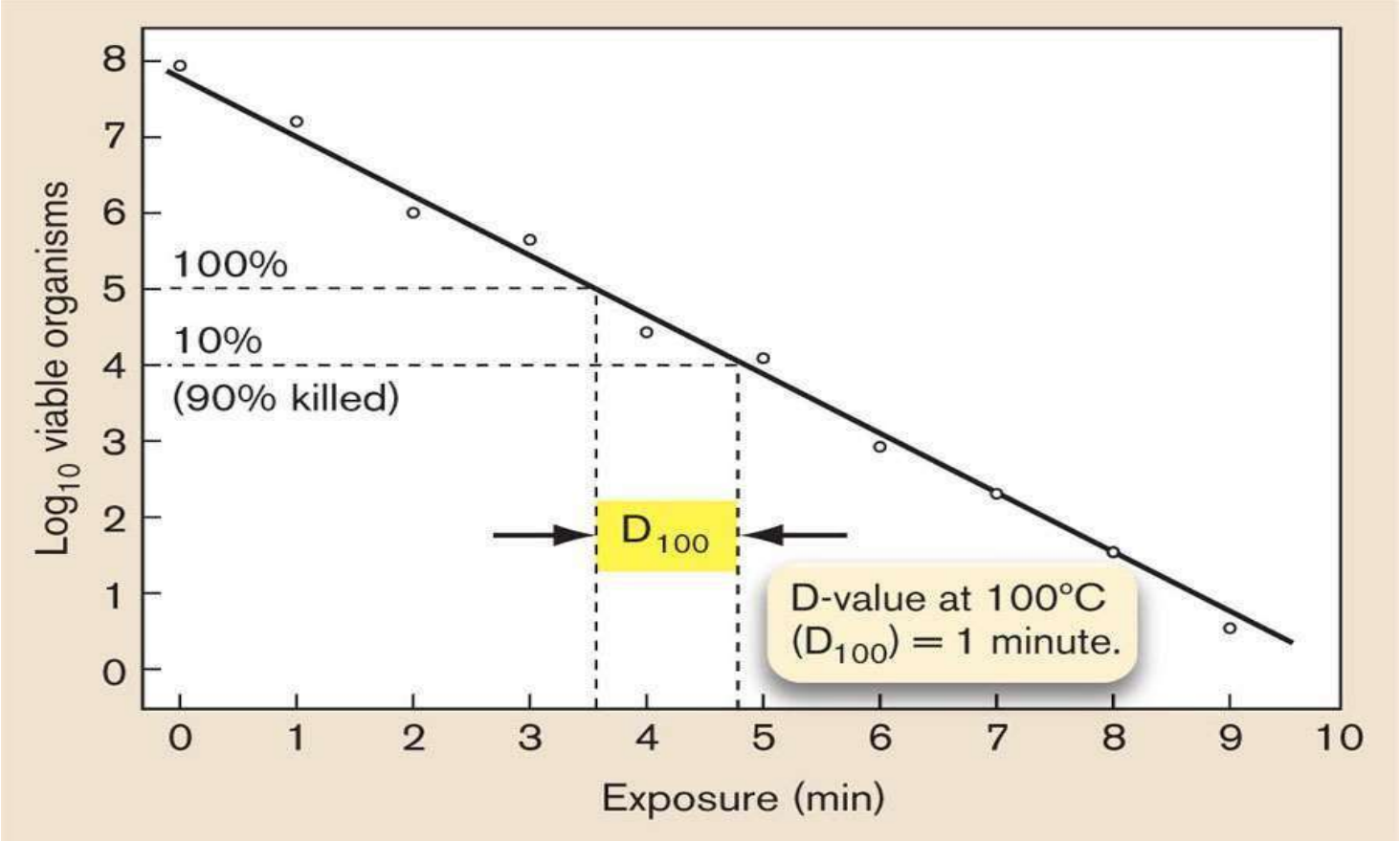
When referring to D-values, for the purpose of thermal analysis, it is proper to give the temperature as a subscript of the "D". For example, given a hypothetical organism which is reduced by 90% after exposure to temperatures of 150° C for 20 minutes, the D-value would be written as D150C = 20 minutes. If generally describing D-value for any temperature, a common abbreviation is DT (where T is temperature) until a value for T is relevant to express, specifically. Another more general abbreviated expression of D-value is D10 (as to denote 10% reduction).

D-value determination is often carried out to measure a disinfectant's efficiency to reduce the number of microbes present in a given environment.

$$D = \mu / \log a - \log b$$ *(mathmaticaly)*

D:decimal reduction time

μ:total exposure time



$$D = T_2 - T_1$$

Effect of temperature depend on several factors:

1-Physical properties of microbial environment

2-Physical state of microbial cell

3-Kind of microbial cell

4-Exposure time

5-Type of heat using ***a-Dry heat*** ***b-Moist heat***

Lab:5

Microbial physiology

Factors effecting Microbial growth (Part 2)

Microbial growth is effected by many factors: some of them are auto factors refer to the genetic material & structure of the cell that determine microbial behavior towards the environment & responsible of microbial variation in nature.

Some of environmental factors directly influence the growth, So they assist in studying m.o. and used efficiently in controlling line particularly in discarding of harmful m.o. especially pathogens species because microbial response differ towards environmental factors e.g: one factor could be lethal & limiting for one species but enhancing the growth of another .

Factors affecting m.o. growth divided into two categories:-

1. Chemical factors
2. physical factors

Sometimes there was interference between physical & chemical factors that can not be separated strictly in so termed physiochemical factors.

physical factors include: -

1. Temperature
2. Hydrogen ion concentration (PH) .
3. Osmotic pressure

Each microbial has its own cardinal temperature that facilitate growth which are:-

1. Optimum temperature

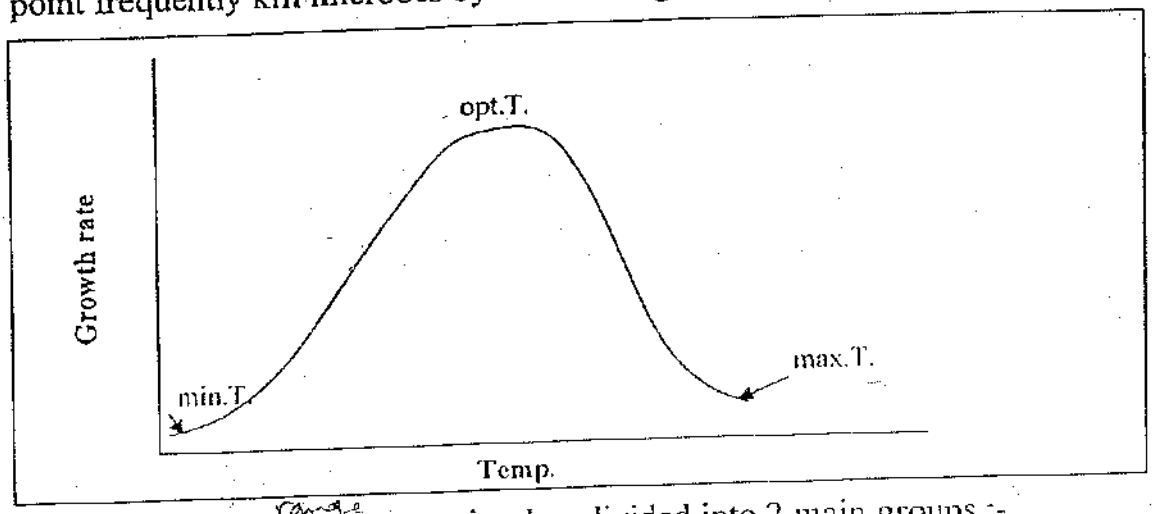
The temperature at which the most rapid rate of multiplication occurs, the microbe shows shortest g.t.

2. Minimum temperature

The lowest temp. at which the microbe grows , all microbe will survive but show negligible growth.

3. Maximum temperature.

The highest temp. at which growth occurs, temp. only slightly above this point frequently kill microbes by inactivating critical enzymes.



According to growth temp. , microbes divided into 2 main groups :-

1. Erythermal : That microbes with broad range of cardinal temp. widely distributed in nature.

2. Stenothermal: That microbes with narrow range of cardinal temp. the limited distributed in the nature.

Bacteria could be divided in to 3 major groups according to its optimum growth temp.

1. Psychrophiles : That microbes capable of growth at (0-20) C°

ex:- Sea bacteria

a. Facultative psychrophiles :

They grow well at temp. in lower mesophiles range.

b. Obligate psychrophiles:

They are limited to low temp. as condition of life .

The importance of psychrophiles is in the degradation (decomposition) of complex organic materials in sea, ocean & cold soils, it also playa role in food spoilage in refrigerated foods.

2. Mesophiles:-

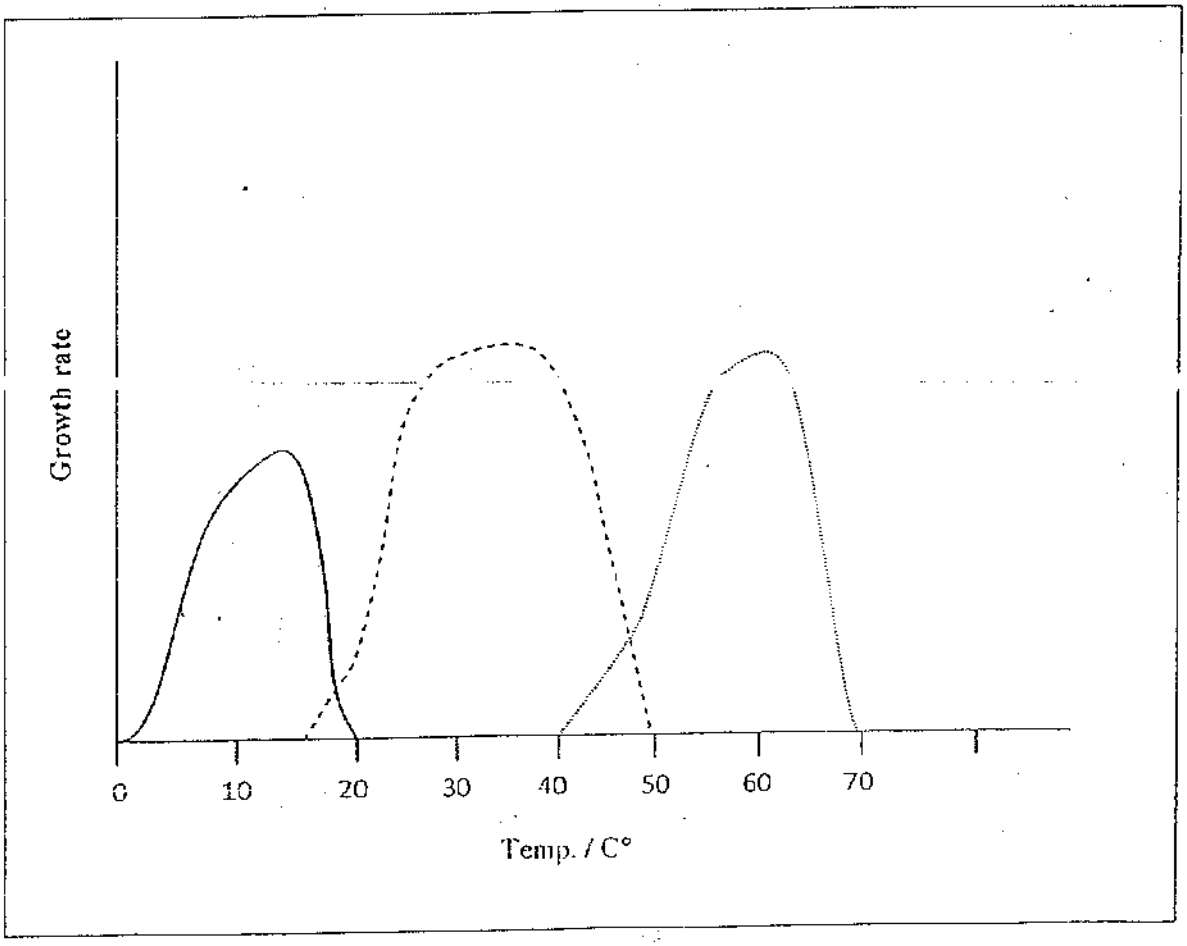
That microbes grow in temp. from (10-40)C°, most known m.o. occur in this group.

3. Thermophiles :-

That m.o. grow best above 50C°.

a) obligate thermophiles :

That m.o. thrive only at high temp. 50C°& above ex : (hot sulfur springs) .



Division of bacteria according to the thermal needs.

stepped a way from the optimum temp. on both directions effects negatively on the growth.

Metabolic enzymes since each enzyme has its own optimum , maximum & minimum temp. , so temp. less than optimum decrease the molecular motion & other physiological functions especially cytoplasmic membrane due to the solidification of lipids & increase of viscosity of proteins resulting in decreasing in enzymatic activity & stop working but does not denaturated (reversible effect) that's why many biological preparations preserved at low temp. (freezing) such as .-proteins , enzymes, viruses ..... etc as lyophilic form.

Temperature over than optimum result in increasing in the rate of enzymatic activities & irreversible denaturation occurs due to breakage of hydrogen bonds &scattering the secondary & tertiary arrangement of proteins plus DNA melting.

Scientists have evaluated the effect of heat on biological activities including growth (the yield of all metabolic activities in the cell) by heat equivalent: speed of reaction of any metabolic process in specific temp. in comparison with its speed in temp. 10 times less than that one

$$Q_{10} = \frac{Kt \div 10}{Kt2}$$

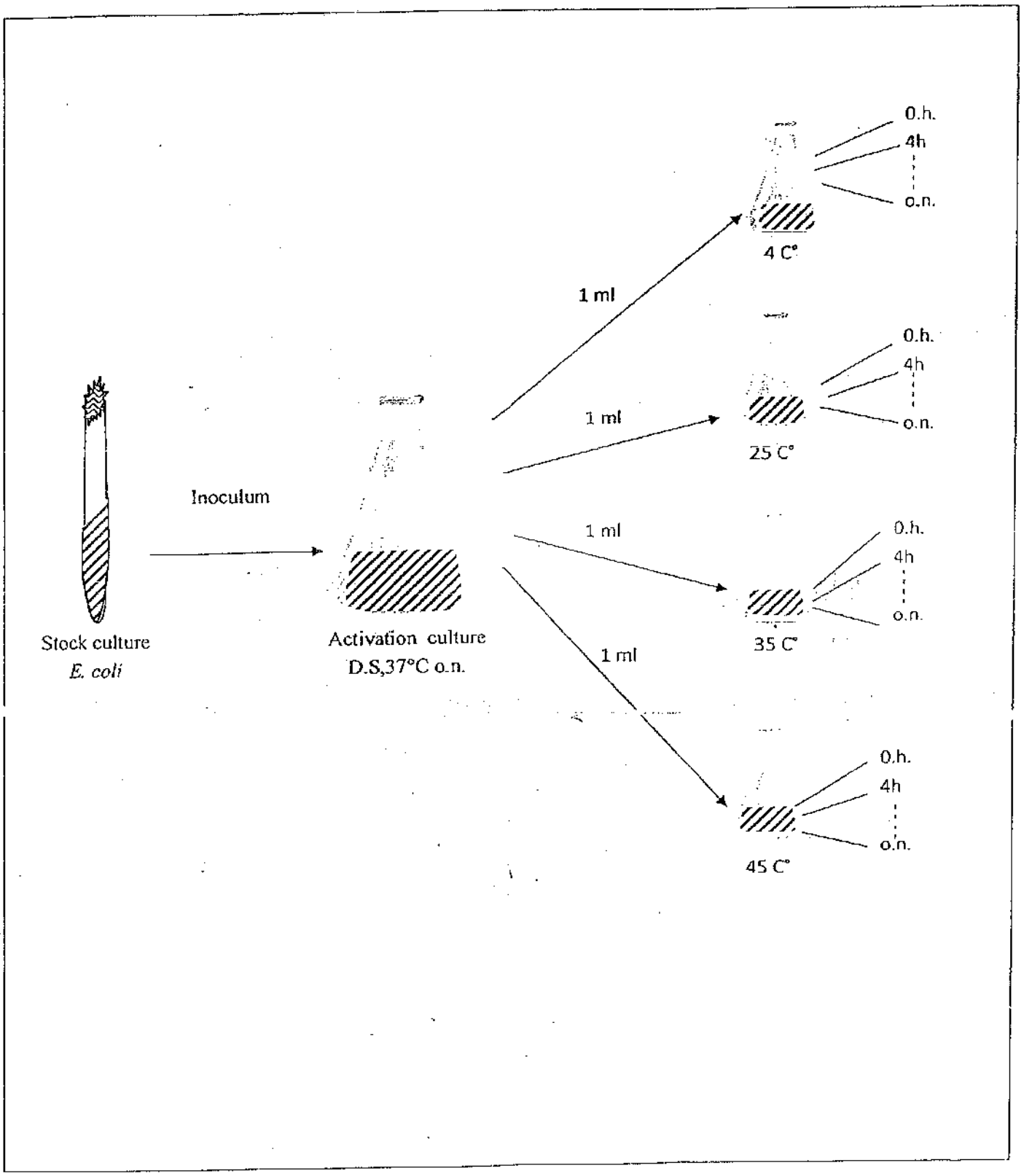
At room temp. (18 -25 CO) → Q_{10} is about (3 - 4) and decreases when temp. increase.

If $Q_{10} > 1$ → growth increase when temp. increase.

If $Q_{10} < 1$ → growth decrease when temp. increase.

If $Q_{10} = 1$ → temp. has no effect on growth.

To determine the Q_{10} of *E.coli* we follow the following scheme :



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فسلجة احياء مجهرية عملي
ثالث بايو
Lab 9

Microbial Physiology

Hydrogen ion concentration (pH)

pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration "expressed in terms of molarity".

$$pH = -\log [H^+] = \log \left(\frac{1}{[H^+]}\right)$$

The pH scale extends from pH 0.0 (1.0 MH^+) to pH 14.0 ($1.0\times10^{-14} MH^+$), and each pH unite represents a ten fold change in hydrogen ion concentration. Microorganisms grow very widely from pH 0 to 2 at the acidic end to alkaline lakes and soil that may have pH values between 9 and 10.

It is not surprising that pH dramatically affects microbial growth. Each species has a definite pH growth range and pH growth optimum:

Acidophiles: have their growth optimum between pH 0 and 5.5.

Neutrophiles: Their growth optimum between pH 5.5 and pH 8.

Alkalophiles: They prefer the pH range of 8.0 to 11.5.

Extreme alkalophiles: have growth optimum at pH 10 or higher.

In general different microbial groups have characteristic pH preferences. Most bacteria are neutrophiles, most fungi prefer more acidic surroundings, about pH 4 to 6, photosynthetic microorganisms also favor slight acidity. Many microorganisms are thrive in extreme pH values for example, the bacteria *Ferroplasma acidarmanus*. Can actually grow at pH 0, or very close to it.

Although microorganisms will often grow over wide ranges of pH and far from their optimum, there are limits to their tolerance, and variations in cytoplasmic pH can harm M.O. that cause disruption of plasma membrane or

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inhibit the activity of enzymes and membrane transport proteins. Changes in the external pH also might alter the ionization of nutrient molecules and reduce their availability to the organisms.

Microorganisms respond to external pH changes using mechanisms that maintain a neutral cytoplasmic pH. Several mechanisms for adjusting to small changes in external pH, the plasma membrane is impermeable to protons. Neutrophiles appear to exchange potassium for protons using membrane transport system, alkalophiles maintain their internal pH closer to neutrality by exchanging internal pH closer to neutrality by exchanging internal sodium ions for external protons.

Other pH maintaining mechanism employed by different bacterial types termed **Decarboxylation** and **Deamination** which occurs in periplasmic space by involving free amino acids, where pH drops. Carboxyle groups splits from free amino acids resulting in elevation of pH again, in case of pH increase to alkaline region. Deamination technique operate in periplasm that split amino groups from free amino acids which reduced pH value.

Microorganisms frequently change the pH of their own habitat particularly in batch culture system by producing acidic or basic metabolic waste products. Fermentative M.O. form organic acids from carbohydrates, other M.O. make their environment more alkaline by generating ammonia through amino acids degradation.

Because M.O. change the pH of their surroundings, buffers often are included in culture media to prevent growth inhibition by large pH changes. Phosphate buffer is a commonly used buffer, buffering capacity of this buffer related by a weak acid ($H_2PO_4^-$) and its conjugate base (HPO_4^-)

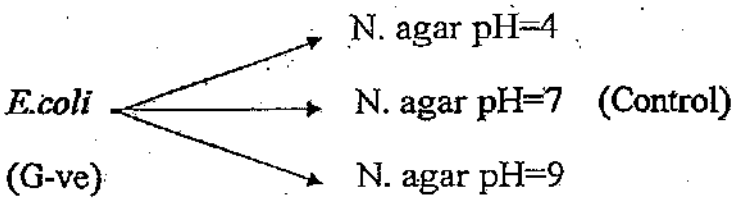
$$H^+ + HPO_4^{2-} \rightarrow H_2PO_4^-$$

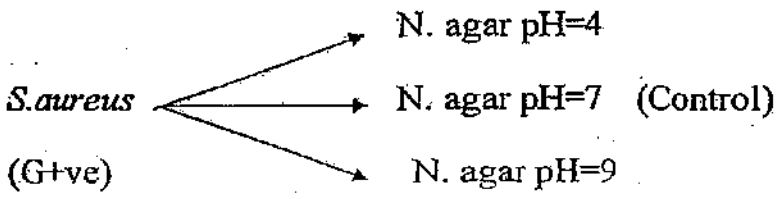
$$OH^- + H_2PO_4^- \rightarrow H_2PO_4^{2-} + HOH$$

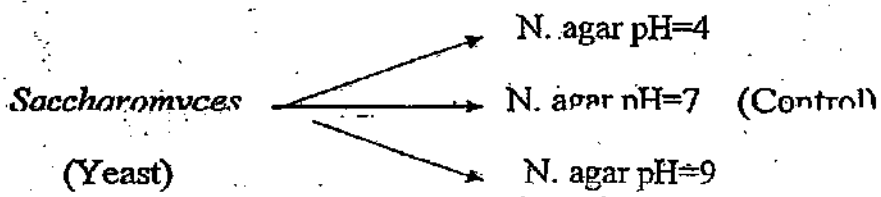
If protons are added to the mixture, they combine with the salt from to yield a weak acid. An increase in alkalinity is resisted because the weak acid will neutralize hydroxyl ions through proton donation to give water, peptides and amino acids in complex media also have a strong buffering effect.

Other used buffers are, acetate-borate, and glycine.

Lab Experiment:-







Incubate at 37°C for 24h. then notice the growth:-

- Good growth.
- Moderate growth.
- Limited growth.
- No growth.

Detergents

Widely industrial proccessable agents that could be used in Lab and domestic purposes, many detergents can be used as disinfectant but others can not.

Detergents can't penetrate the microbial cells, they adsorbe on their outer surfaces leading to change in cells permeability of entrance and exit materials due to surface tension reduction of liquid surrounding the microbial cells.

The detergent molecule is bipolar consist of two portions :-

1- Lipophilic Portion
   Also called lipid soluble, uncharged part consist of long hydrocarbon chains (or alkyl groups), effectiveness of these detergents depend on:-
   a- The length of these chains.
   b- The unsaturated state of these chains.
2- **Hydrophilic portion**
   Water soluble, charged part of the detergent, this charge part, detergents can be divided into (-) and (+).

Mechanism of action of detergents

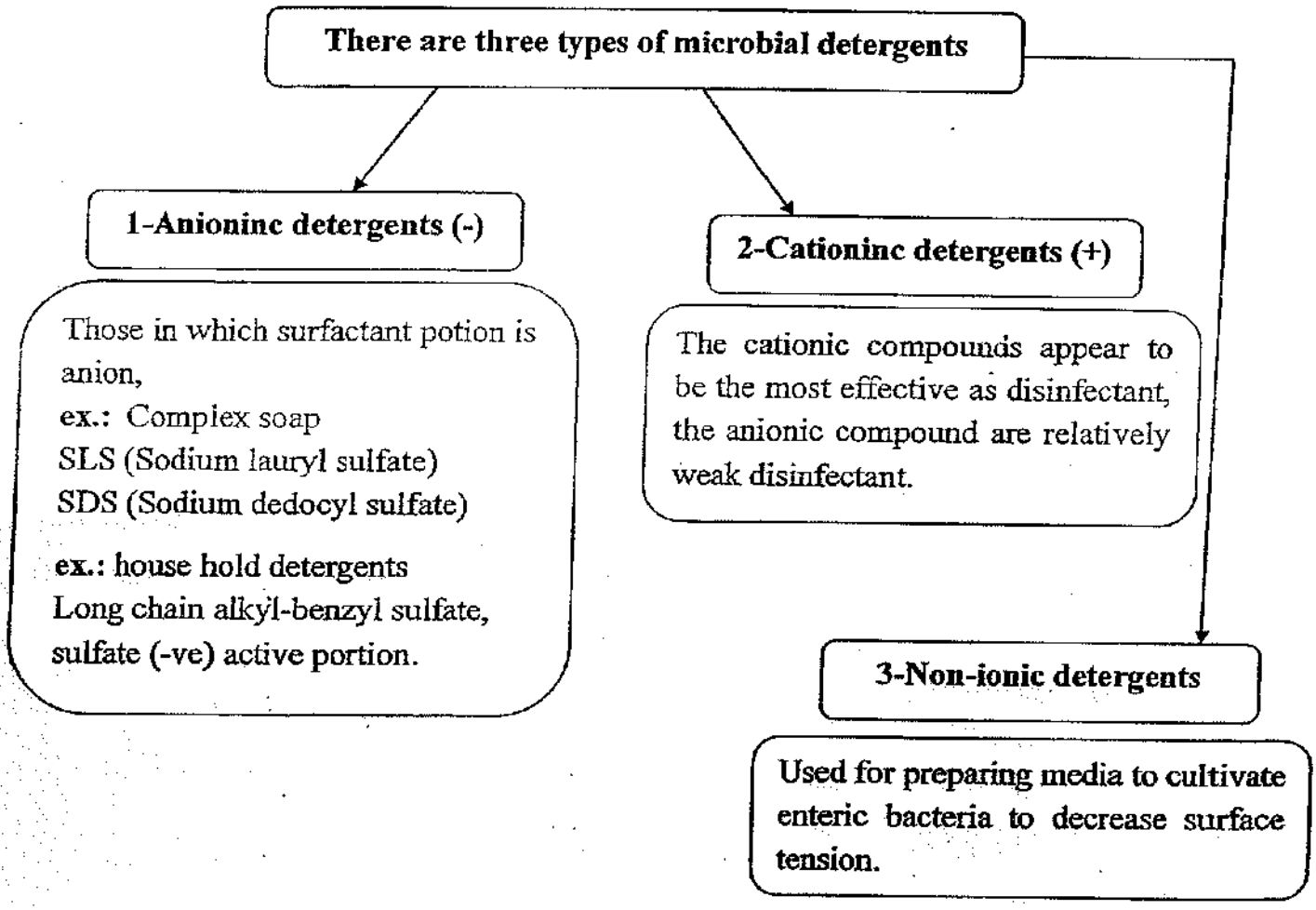
When detergents are dissolved in aqueous solutions, the lipophilic part of the detergent will react with microbial lipids or with lipids droplets in dirt, while the hydrophilic part lay away from microbial cells dirt particles and lay along the interface space between aqueous solutions and microbial cells or dirt particles.

As a result to this lineup the surface tension of aqueous solution will decrease ↓ resulting in rupture of cell membranes rich in fats due to milking of outer membranes → materials will influence outside the cell.

Rupture will gets contact between cellular proteins aggregates shipped to the detergent resulting in denaturation of these proteins and killing, so

killing by detergents is not only by chemical action but mechanical action also.

Detergent solutions are called foam (sudsing agent) + bubbles containing solvents considered good surfaces to adhere dirts and microbial cells that can be removed by repeated rinsing.



Generally bacteria prefer surface tension 50dyne/cm except *Enterobacter* prefer low surface tension 38 dyne/cm because it grow in intestine which found lipids milking by bile salts that cause decrease of surface tension of intestine.

Tween 80 or Triton X-100, uncharged materials, does not cause side reaction but lipid milking result in decreasing surface tension.

Evaluation of detergents

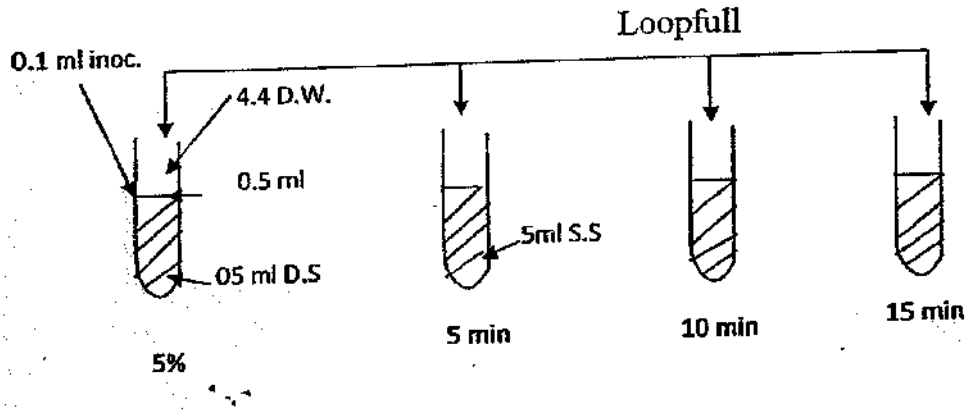
Two parameters should be estimated:-

1- Treatment time.
2- Concentration of detergent.

Evaluation of detergent by :-

1- Tube Dilution method T.D.M.
2- Use dilution test U.D.T.

Conc. %	D.S. medium	Detergent test	D.W.	inoculum	Total volume
5%	5ml	0.5ml	4.4 ml	0.1 ml	10 ml
10%	5ml	1 ml	3.9 ml	0.1 ml	10 ml



Incubate at 37°C for 24h, results recording depends on turbidity.
((+) turbid tubes (-) clear tubes)- means that detergent is not effective to kill M.O in that concentration during that time (usually 10 min not 5 min).

Q/ Prepare the following concentrations of Dettol against *S. aureus* in total volume 10 ml? (20%,50%,70%)

Antimicrobial Action of Some Chemical agents

Antimicrobial agents are chemicals that kill or inhibit the growth microorganisms. Antimicrobial agents include chemical preservatives and antiseptics, as well as drugs used in the treatment of infectious diseases of plants and animals. Antimicrobial agents may be of natural or synthetic origin, and they may have a static or cidal effect on microorganisms.

Germistatic: agents that inhibit the growth of M.O. by removed M.O. or resume their growth.

Germicides: agents that kill M.O. but not necessarily bacterial endospores.

Types of antimicrobial agents

Antiseptics: microbicidal agents harmless enough to be applied to the skin and mucous membrane; should not be taken internaslly. Examples: mercurials, silver nitrate, iodine solution, alcohols, detergents.

Disinfectants: Agents that kill microorganisms, but not necessarily their spores, not safe for application to living tissues; they are used on inanimate objects such as tables, floors, utensils, etc. Examples: chlorine, hypochlorites, chlorine compounds, lye, copper sulfate, quaternary ammonium compound.

Preservatives: static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic. Examples; calcium propionate, sodium benzoate, formaldehyde, nitrate, sulfur dioxide. Table 2 is a list of common preservative and their uses.

Detergents

A detergent is a surfactant (a surface – active substance) or a mixture of surfactants having cleaning properties in dilute solutions. Synthetic detergents have similar molecular structures and properties as soap. Although the cleansing action is similar, the detergents do not react as readily with hard water ions of calcium and magnesium. Detergent molecular structures consist of a long hydrocarbon chain and a water soluble ionic group. Most detergents have a negative ionic group and are called anionic detergents. The majority are alky sulfates. Others are surfactants which are generally known as alkyl benzene sulfonates.

Anionic detergents

Typical anionic detergents are alkylbenzenesulfonates. The alkylbenzene portion of these anions is highly lipophilic and the sulfonate is the hydrophilic component.

Cationic Detergents:

Another class of detergents have a positive ionic charge and are called "cationic" detergents. In addition to being good cleansing agents, they also possess germicidal properties which makes them useful in hospitals. Most of these detergents are derivatives of ammonia.

A cationic detergent is most likely to be found in a shampoo or clothes "rinse". The purpose is to neutralize the static electrical charges from residual anionic (negative ions) detergent molecules. Since the negative charges repel each other, the positive cationic detergent neutralizes this charge.

Neutral or non-ionic detergents:

Nonionic detergents are used in dish washing liquids. Since the detergent does not have any ionic groups, it does not react with hard water ions. In addition, nonionic detergents foam (mass of small bubbles) less than ionic detergents. The detergent molecules must have some polar parts to provide the necessary water solubility.

Bile Salts - Intestinal Natural Detergents:

Bile acids are produced in the liver and secreted in the intestine via the gall bladder. The bile salt is called sodiumglycoholate. Another salt can be made with a chemical called taurine.

The main function of bile salts is to act as a soap or detergent in the digestive processes. The major action of a bile salt is to emulsify fats and oils into smaller droplets. The various enzymes can then break down the fats and oils.