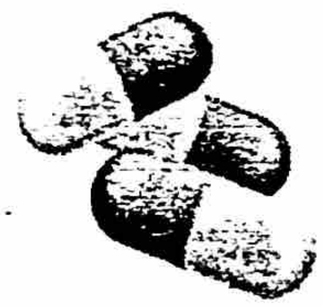
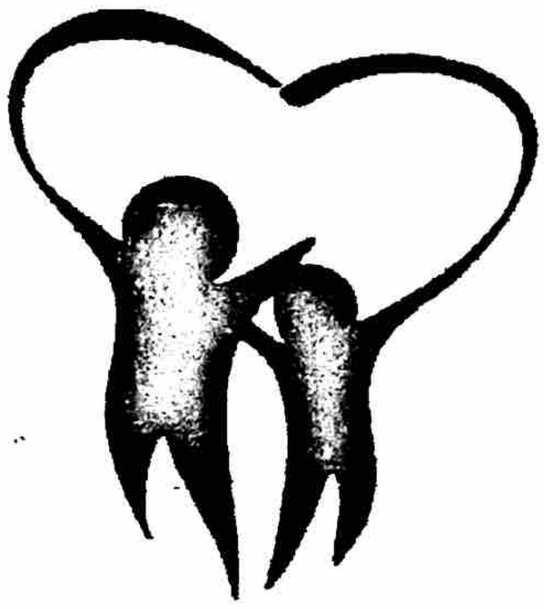
2nd-Exam..

Major :Medicine & Dintist

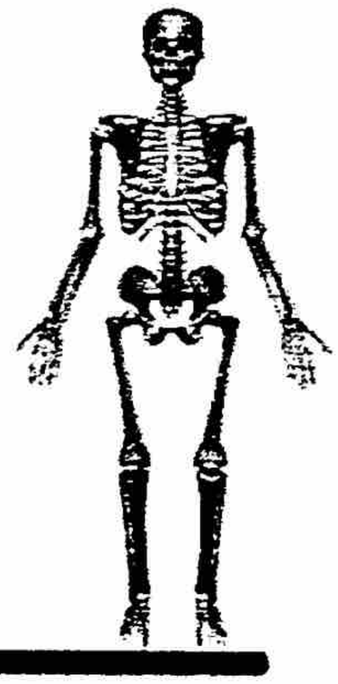
Lecture :Micro #1

Lecturer : Dr.Hussain Al Qasim





# TAQAROB



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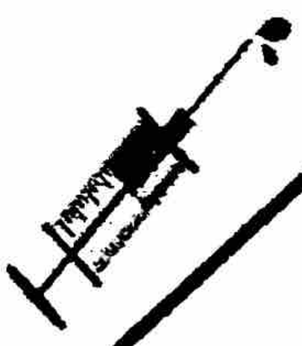

مدير الأكاديمية :محمود ابوعيد

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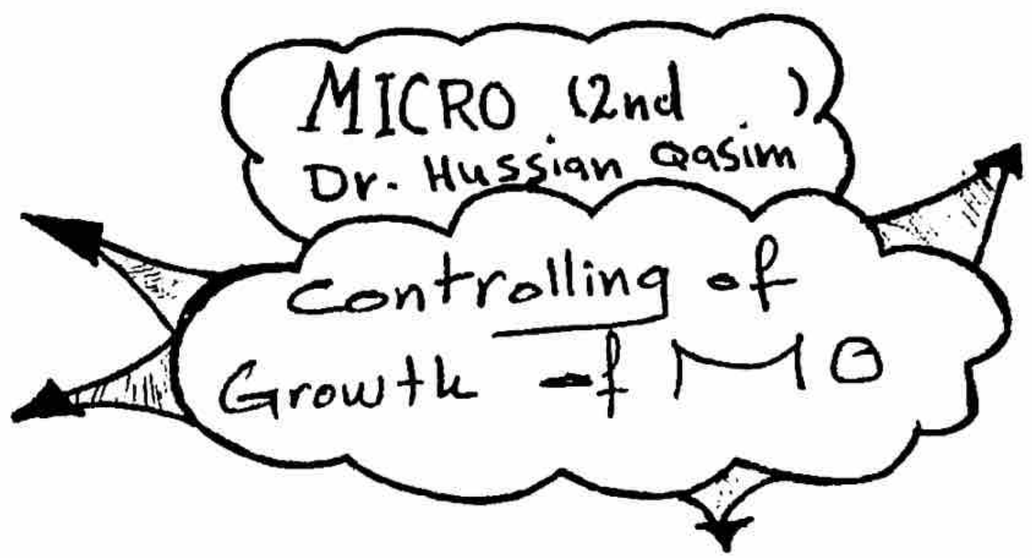
* in vivo = inside body
* in vitro = outside body

* Microbial Growth → increase in number of cells (not in size)
* Bacterial growth = Binary Fission
* Generation time = time needed to duplicate the number of MO

* Generation time of MO

| Majority | Minority |
|---|---|
| - short<br>- rapid grower<br>- report after 24 hours | - Long<br>- slow grower<br>- report after 4 weeks<br>- Ex = MTB |




* Growing Microbes accumulate into colonies مستعمرات → group of cells large enough to be seen without a microscope !!

* Colony = hundreds of thousands to billions of cells

* Factors Influencing Microbial Growth

1- Temperature 2- Moisture
3- osmotic Pressure
4- pH
5- Barometric pressure
6- Gases 7- Radiation
8- Chemicals
9- Presence of neighboring microbes

* Requirements of Microbial growth

| Chemical | physical |
|---|---|
| source of → carbon nitrogen sulfur. | Temp. pH osmotic P. |

[1] Temperature

a- The minimum growth temp. → the lowest temp. at which MO can grow
b- optimum growth temp. → the temp. at which MO grows best
c- The maximum growth temp. → the highest temp. at which growth is possible

[2] pH (acidity or alkalinity)

- Most bacteria grow blw pH 6.5 - 7.5 (near neurality)

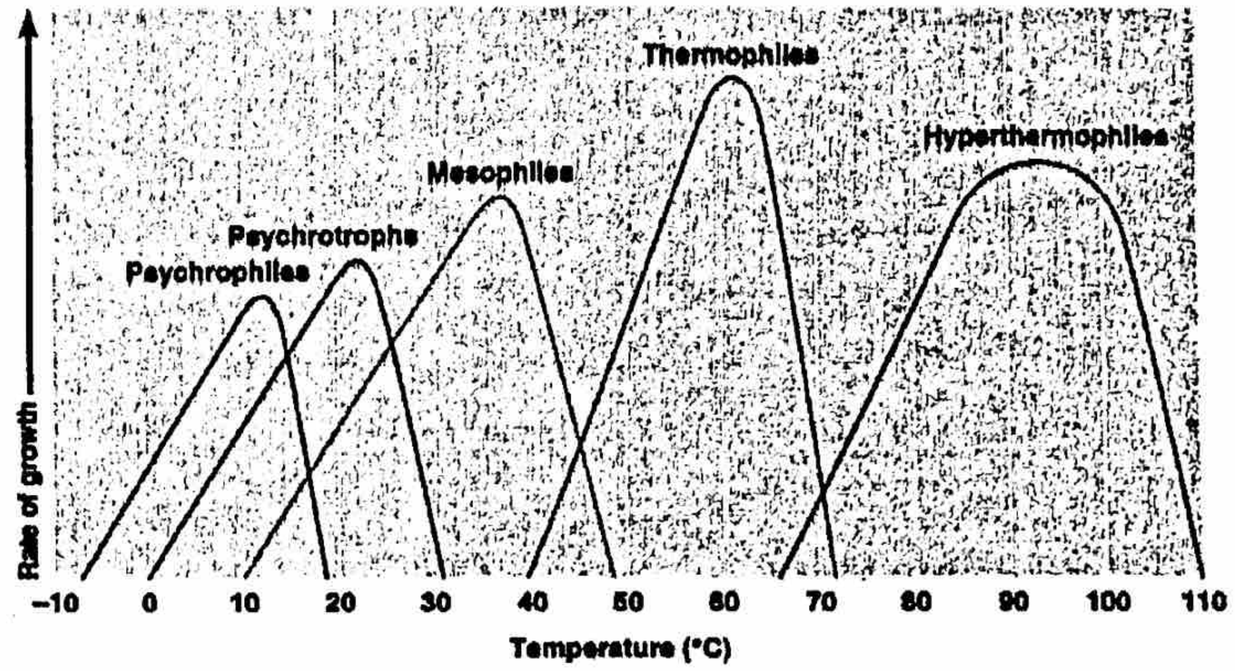
- Acidophiles → grows in acidic environment → Examples:
  a- MTB b- Nocardia
  c- Rhodococcus d- Fungi → Yeast / mold
  pH = 5-6

- Alkaliphiles → grows in alkaline environment (pH > 8.5)
  Ex = Vibrio Cholera

- Alkalinity inhibits MO growth but rarely used to preserve Food

| Microorganism | minimum | Optimum | maximum |
|---|---|---|---|
| Psycrophiles | -10 | 12 | 20 |
| Psycrotrophs | 0 | 22 | 30 |
| Mesophiles | 10 | 37 | 50 |
| Thermophiles | 40 | 62 | 70 |
| Hyperthermophiles | 65 | 92 | 110 |

Most pathogens and indigenous microflora are mesophiles





a- obligate aerobes require an atmosphere containing molecular oxygen in concentrations comparable to that found in room air (20%–21% O2) → grow on surface

b- acultativeanaerobesare capable of surviving in either the presence or absence of oxygen; anywhere from 0% O2 to 20% to 21% O2 → grow on the surface , at the middle of tube & at the bottom

c- obligate anaerobeis an anaerobe that can only grow in an anaerobic environment (an environment containing no oxygen) → grow at the bottom

d- aerotolerant anaerobe does not require oxygen, grows better in the absence of oxygen, but can survive in atmospheres containing molecular oxygen →(prefer the bottom but can tolerate the surface)

e- microaerophiles require reduced oxygen concentrations (usually around 5% oxygen) → grow in the middle of the tube

[3] osmotic pressure

- MO obtain almost all their nutrients from the surrounding water
- MO require water for growth → are made up of 80-90% water
- Halophile = MO prefers ↑ salt concentration.

* Extreme / obligate halophiles
→ MO that have adapted so well to ↑ salt conc. that they require for growth (Dead sea)

* Facultative halophiles → don't require ↑ salt conc., but are able to grow at salt conc. of 2-15 %

Controlling growth of MO →

[5] organic growth Factors

- essential compound = organism is unable to synthesize & must be obtained from environment
- Ex. ] vitamins, amino acids, purines, pyrimidines

* Chemical requirements
- Carbon - Nitrogen
- Sulfur - phosphorus
- potassium - Magnesium
- calcium - oxygen

[4] oxygen

a- obligate aerobes → require O2 comparable to room air (20-21%)

b- Microaerophiles → require reduced O2 con. ~ 5%

c- obligate anaerobe → can only grow in an anerobic environment (0% O2) in

d- Aerotolerant anaerobe → grows better in absence of O2 but can surive in presence of O2

e- Facultative anaerobe → Capable of surviving in either presence or absence of O2 (0% - (20-21)%)

* Anaerobic culture media
- anaerobic media = Reducing media
- contain → Sodium thioglycolate that combine O2 & deplete O2 in medium
- special transparent jars are used to grow anaerobic on petri plates to observe colonies
- Culture Plates are placed in the Jar & O2 is removed by addition of Packet of chemicals which react with O2 to form water.

# *Culturing bacteria in the lab

## *Bacterial growth →

→ growth: multiplication of bacteria

→ bacterial growth → increas in the number of organisms rather than an increase in their size

→ generation time → time taken by bacteria to undergo binary fission

- short GT → rapid growers
- long GT → slow growers

## Growth in Vitro

### Culture Media

* media used in microbiology lab to culture bacteria → artificial media or synthetic media → don't occur naturally

* one way to classify culture media is based on whether the exact contents of the media are known.

#### * Liquid medium → known as Broths

→ contained in tubes → tubed media

#### * Solid medium → prepared by adding agar to liquid medium & then pouring into tubes or Petri dishes where the media solidify.

#### ** Agar → complex polysaccharide obtained from red marine algae

#### * chemically defined medium →

→ one in which all the ingredients are known

→ prepared in the lab

→ contents → CHO, AA → salts

#### * Complex medium → one in which the exact contents are not known

→ contain digested extracts

→ from animal organs, fish, yeast & plants

#### * Fastidious → MO that are difficult to grow in the lab & have complex nutritional requirements

#### * obligate intracellular Pathogens →

won't grow at all on artificial culture

→ must be inoculated into live animals, embryonated chicken eggs or cell cultures.

Ex] viruses, rickettsias, Chlamydia

#### * other MO

→ won't grow on artificial media

→ Ex] T. pallidum, M. leprae

# * Culture Media *

## [1] Enriched medium

- contains rich supply of nutrients
- Broth or solid
- promotes growth of Fastidious MO
- Examples

[a] Blood agar (Red)
= Red nutrient agar + 5% sheep RBC
* contains hemoglobin

[b] chocolate agar (Brown)
= nutrient agar + powdered hemoglobin (Hb)
* more Hb than blood agar

* Both are used with MO that don't grow on blood-free agar
* chocolate agar = For] H. influenzae, N. gonorrhoeae
don't grow on blood agar

## [2] Selective Media

- solid media to discourage growth of unwanted MO + encourage growth of wanted MO

*Examples

[a] Bismuth sulfite agar → to isolate S. typhi from feces

[b] Sabouraud's Dextrose agar → pH = 5.6 → to isolate Fungi

[c] MacConkey agar → selective for Gram -ve (⊖ G+ve)

[d] phenylethyl alcohol PEA

[e] Colistin nalidixic acid CNA
d+e] → ⊖ growth of G-ve

[f] Thayer-Martin agar

[g] Martin-Lewis agar
Chocolate agar + extra nutrients + antimicrobial agents
→ selective for N. gonorrhea.

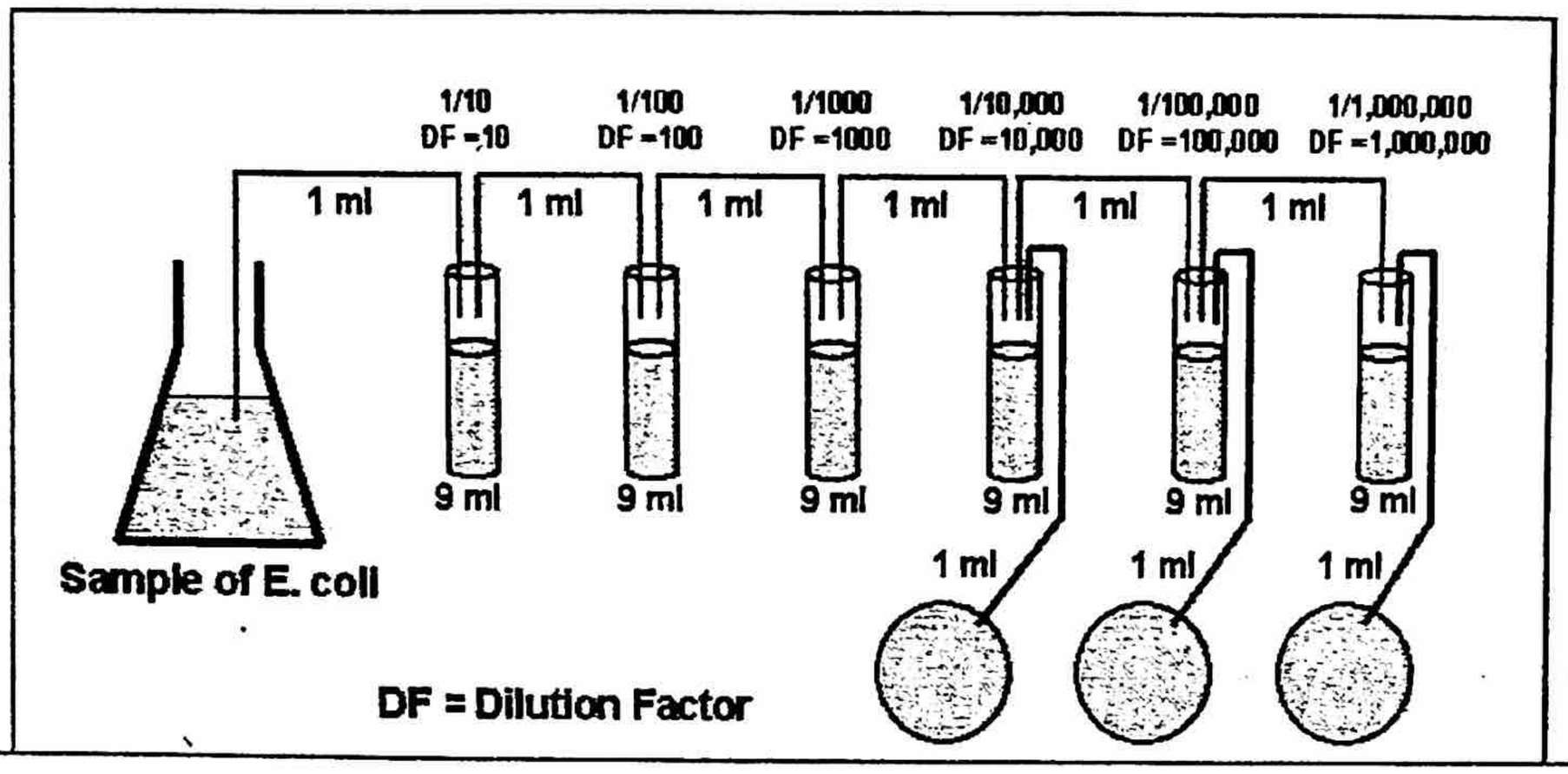
## [3] Differential Media

- Solid
- allows to differentiate blw MO
- Examples

[a] Mannitol salt agar
→ 7.5% NaCl → for S. aureus (mannitol Fermenter)
*pink medium → yellow

[b] MacConkey agar → to distinguish blw different G-ve bacteria
→ Lactose Fermenter: pink colony
→ non-lactose Fermenter: colorless Colony

[c] Blood agar → to determine type of hemolysis

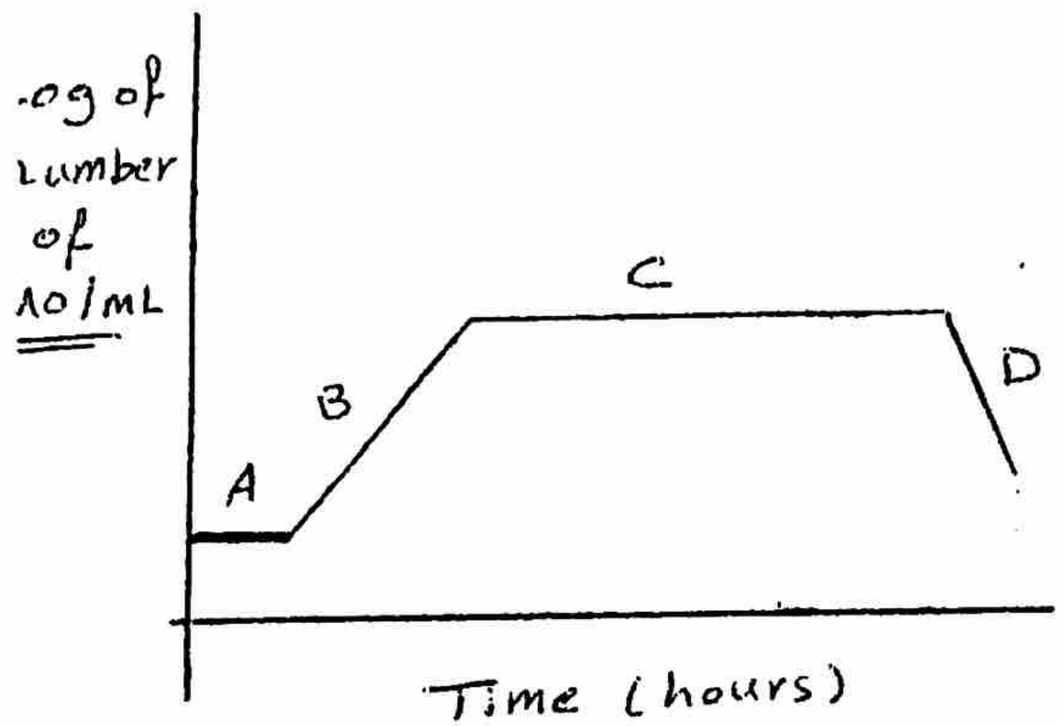



**Direct Measurements of Microbial Growth**

- **Plate counts:** Perform serial dilutions of a sample
  - Advantage: it measures the number of viable cells
  - Disadvantage: it takes some time, about 24 hours.

1) Take 6 dilution tubes, each containing 9 ml of sterile saline.
2) Dilute 1 ml of a sample by withdrawing 1 ml of the sample and dispensing this 1 ml into the first dilution tube.
3) Using the same procedure, withdraw 1 ml from the first dilution tube and dispense into the second dilution tube. Subsequently withdraw 1 ml from the second dilution tube and dispense into the third dilution tube. Continue doing this from tube to tube until the dilution is completed.
4) Transfer 1 ml from each of only the last three dilution tubes onto the surface of the corresponding agar plates.
5) Incubate the agar plates at 37°C for 48 hours.
6) Choose a plate that appears to have between 30 and 300 colonies.
7) Count the exact number of colonies on that plate
8) Calculate the number of CFUs per ml of original sample as follows:

CFUs per ml of sample = The number of colonies X The dilution factor of the plate counted




## A) Lag phase

* bacteria absorb nutrients
* synthesize enzymes
* prepare for cell division
* bacteria don't increase in number

## B) Log phase

* bacteria multiply rapidly
* growth rate is the greatest
* always brief
* steeply sloped straight line
* also called :-
  * Logarithimic growth phase
  * Exponential growth phase

**Bacterial Population curve**

## C) Stationary Phase

* nutrients in the medium are used up
* conc. of toxic waste products build up
* Rate of division slows
* number of dividing bacteria equals the number that are dying
* culture is at its greatest population density.

## D) Death Phase

* overcrowding occurs
* ↑ toxic waste products
* ↓ nutrients
* MOs die at a rapid rate
* if spore-former → production of spores
* some cells look different from healthy organisms
* morphologic changes in the cells may appear

* some cells undergo involution & assume various shapes

* also called → Decline phase

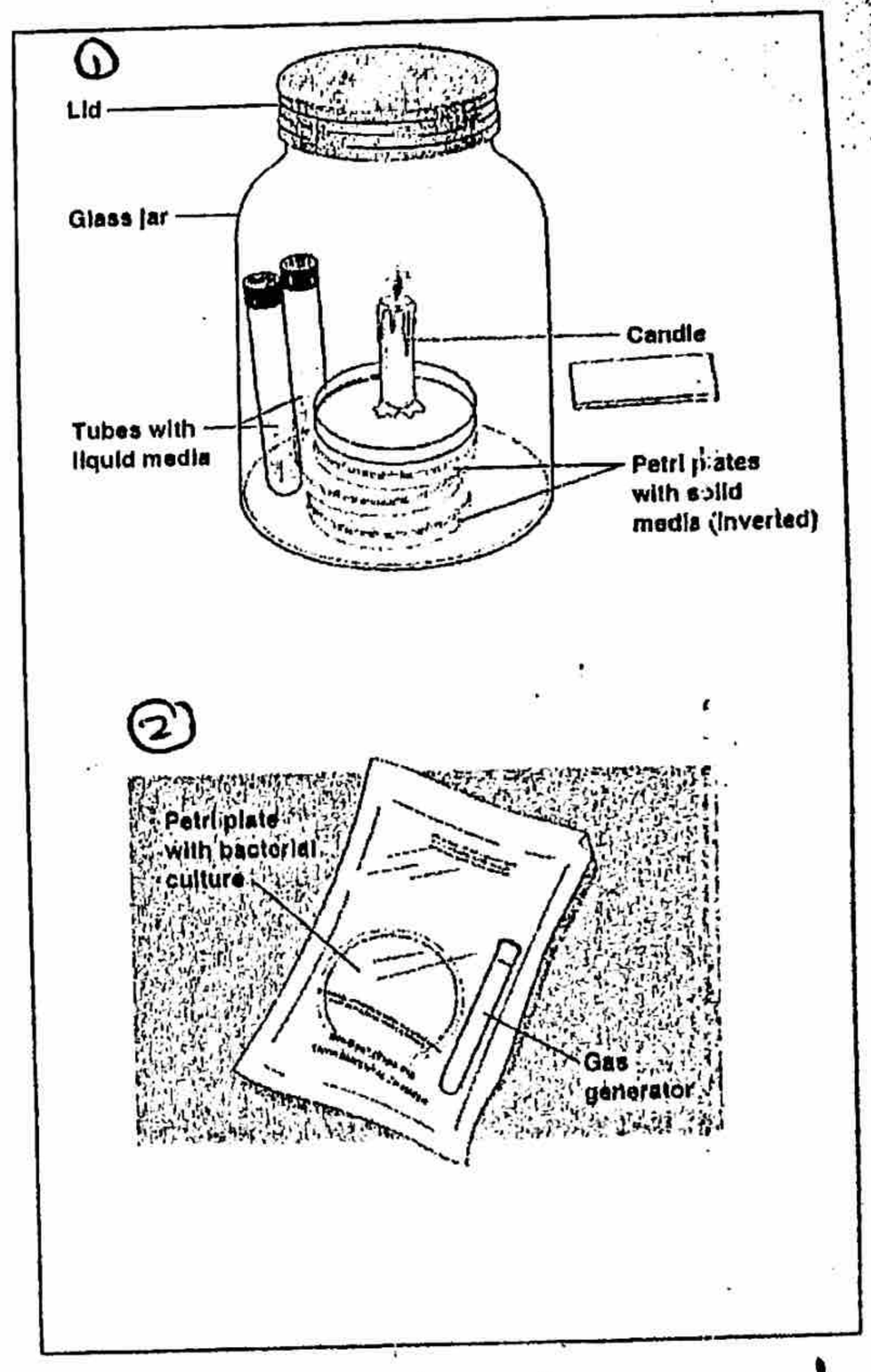
* Capnophiles → MO grow better at high $CO_2$ Concentration

**TABLE 6.2** A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

| Constituent | Amount |
|---|---|
| Glucose | 5.0 g |
| Ammonium phosphate, monobasic ($NH_4H_2PO_4$) | 1.0 g |
| Sodium chloride (NaCl) | 5.0 g |
| Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) | 0.2 g |
| Potassium phosphate, dibasic ($K_2HPO_4$) | 1.0 g |
| Water | 1 liter |

**TABLE 6.1** Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

| Constituent | Amount |
|---|---|
| Peptone (partially digested protein) | 5.0 g |
| Beef extract | 3.0 g |
| Sodium chloride | 8.0 g |
| Agar | 15.0 g |
| Water | 1 liter |




1 Candle jar → Cultures are placed in a sealed jar containing a lighted candle → consumes $O_2$ + produces $CO_2$

2 $CO_2$ packet → Consists of a bag containing a petri plate + $CO_2$ generator

# The Control of Microbial Growth

Sepsis = Microbial Contamination

Asepsis = Absence of significant contamination

Aseptic surgery techniques = prevent microbial contamination of surgical wounds

Sterilization = Removal of all forms of microbial life including endospores by → Heating, Filtration, Gases

Disinfection = Removal of vegetative (non spore forming) pathogens by → chemicals → boiling water; UV radiation → steam

commercial sterilization = heat treatment to kill endospores of clostridium botulinum in canned foods

↓

Note = more resistant endospores of thermophilic bacteria may survive but won't germinate under storage conditions.

Antisepsis = Removal of vegetative pathogens on living tissues

Antiseptic = chemical used for Antisepsis

Sanitization = lower microbial counts on eating utensils

Degerming = Removal of microbes from a limited area ] by alcohol-soaked swab.

Biocide / Germicide = kill microbes

Bacteriostatic = inhibiting growth of microbes.

# Physical methods of Microbial Control

## I Heat :

- Kills MO by denaturing their enzymes & changing 3D shapes
- leads to functional inactivation of enzymes
- sterilization of → Canned-Food, Glass-ware, hospital-Instrument
- Thermal Death Point (TDP) → lowest temp. at which all cells in a Liquid culture are killed in 10 minutes
- Thermal Death Time (TDT) → minimal time length to kill all bacterial cells at a given temp.

### A Moist Heat

- Protein Coagulation (denaturation)

#### (i) Boiling or flowing steam

- Kills vegetative bacteria & fungi & almost all viruses within 10 min
- less effective on endospores
- uses: Dishes, basins, pitchers

#### (ii) Autoclaving

- steam under pressure
- the higher the pressure, the higher the temp.
- Pressure = 15 pounds
- temp. = 121 °C
- All MO & endospores are killed in 15 minutes
- uses: culture media, solutions, utensils, dressings, IV equipments, syringes

### B Pasteurization

- Kills all Pathogens & most non-pathogens
- Classical = 63°C for 30 min
- ↑ temp. = short time → 72°C / 15 sec
- ultra ↑ temp. = 140°C / < 1 sec
- Thermoduric MO → survive pasteurization, but unlikely to cause disease or spoilage of refrigerated milk.
- uses: milk, cream, beer, wine

### C Dry Heat } very effective in sterilization

#### (i) Direct Flaming → burning contaminant to ashes

- uses: paper cups, bags, contaminated dressings, wipes, animal carcasses

#### (ii) incineration → burning to ashes

- Note: direct flaming → used in inoculating loops

#### (iii) Hot air sterilization → requires temp. of 170°C for about 2hr

- uses: empty glass-ware, instruments, needles, glass syringes

# Physical methods of Microbial Control

## [2] Filtration : remove microbes
- Passage of liquid or gas through a screenlike material with pores small enough to retain MO.
- to sterilize heat-sensitive materials
  - some culture media
  - vaccines
  - enzymes
  - antibiotic solutions
- membrane Filters = substances such as cellulose esters or plastic polymers
- Pore sizes = 0.2 µm & 0.45 µm

## [3] Cold
- mechanism :
  - ↓ chemical reactions
  - possible changes in protein
- uses] Food, drug & culture preservation.

### [A] Refrigeration ] Bacteriostatic

### [B] Deep-Freezing
- temp = -50 - -95°c
- preserving microbial cultures

### [C] Lyophilization
- Most effective method for long-term preservation of microbial cultures
- Freeze-drying
- MO suspension quickly frozen at -54 to -72 & the water is removed by vaccum (sublimation)

### [A] Ionizing Radiation ]
- X-rays
- γ-rays
- electron beams
- wavelength shorter than non-ionizing
- more energy than non-ionizing
- ionization of $H_2O$ → hydroxyl radicals
- sterilization of pharmaceuticals & disposal dental supplies.

### [B] Non-Ionizing Radiation ] UV-light
- not very penetrative
- Kill microbes in the air, hospital rooms, nurseries, operating rooms, cafeterias
- to disinfect vaccines.

Note = Microwaves → Kill by moisture heating ↻.

## [6] osmotic pressure
- ↑ con. of salts & sugars to preserve food.
- Causes plasmolysis of MO
- salt → to preserve meats
- sugars → " " fruits
- Yeasts & molds are capable of growing under ↑ conc. & ↑ osmotic P.

## [7] Radiation
- DNA damage

## [H] High Pressure
- Proteins & CHO alteration
- Preservation of colors, flavors & Fruit juices

## [5] Dessication
- disruption of metabolism by loss of water
- Food preservation
- MO can't grow or reproduce without water but can remain viable for years.

## Chemical Methods of Microbial Control

### 1 Phenol & phenolics
- mechanism → disrupt plasma m.
  → denature enzymes

**A Phenol**
- rarely used
- irritating & bad odor

**B Phenolics**
- Ex: O-Phenylphenol
- uses: environmental surfaces, instruments, skin surfaces, mucous membranes

**C Cationic detergents**
- Quaternary ammonium compounds
- enzyme inhibition
- Protein denaturation
- disruption of plasma m.
- antiseptic for skin, instruments utensils, rubber goods
- bactericidal / bacteriostatic Fungicidal / virucidal against enveloped viruses.

### C Bisphenols
- disinfect handsoaps & skin lotions
- Ex: Triclosan — broad spectrum, more effective against G+ve

### 2 Biguanides (chlorhexidine)
- disruption of plasma m.
- skin disinfection
- bactericidal to G+ve G-ve
- non-toxic

### 6 surface active agents

**A Soaps & acid-anionic detergents**
- mechanical removal of microbes by scrubbing
- skin degerming

**B Acid-anionic detergents**
- Sanitizers in dairy & Food-processing industry
- non-toxic - Fast-acting
- non-corrosive.

### 3 Halogens (Iodine & chlorine)
- Iodine = ⊖ Protein function
  = strong oxidizing agent
- chlorine = Forms → hypochlorous acid → alters cellular components [strong oxidizing agent]
- Iodine = antiseptic
- chlorine = water disinfectant

### 4 Alcohol
- Protein denaturation & Lipid dissolution
- disinfectant for thermometers & skin before injection.
- bactericidal & Fungicidal
- not effective against:
  - endospores
  - nonenveloped viruses
- Ex: Ethanol, Isopropanol

### 5 Heavy metals [silver, mercury]
- proteins & enzymes denaturation
- biocidal
- silver nitrate = to prevent gonorrheal ophthalmia neonatorum
- mercurochrome = disinfect skin & mucous membrane.
- Copper sulfate = algicide

7 organic acids
- metabolic inhibition
- mostly affecting molds
- widely used to control molds of some bacteria in foods of cosmetics

8 Aldehyde
- Protein denaturation
- very effective antimicrobial
- Glutaraldehyde → less irritating than Formaldehyde → disinfection of medical equipments.

9 Gaseous sterilants
- Ex = Ethylene oxide
- for objects damaged by heat

10 peroxygens (ozone)
- oxidizing agents
- for contaminated surfaces of deep wounds
- $H_2O_2$ = poor antiseptic good disinfectant

Chemical Methods of Microbial control

* Lister :→ The 1st to use Phenol to control surgical infections in the operating rooms.

- Most chemical agents are disinfectants that reduce microbial populations to safe levels

* principles of effective disinfection

A conc. of disinfectant =
↑ Con. = ↑ activity

B organic matter =
cleaner object = ↑ activity

C pH → different from MO to another

D Time
↑ time = ↑ activity

E Temp. → different from MO to another

F Type of microbes, number of microbes

G presence of spores

* Thermophiles = non pathogenic

* Placing a cell in

| Hypertonic solution | Hypotonic solution "distilled water" |
|---|---|
| - shrinkage | - swelling ] plasmoptysis |
| - plasmolysis | - Burst ] |

* SS-agar = Salmonella-Shigella agar → selective media

* Preserving bacterial cultures

- Refrigeration = short-term storage
- for long-term preservation

ⓐ Deep-Freezing = Cultures placed in a suspending liquid & quick-frozen at -50 to -95°

ⓑ Lyophilization = (Freeze-drying) suspension of microbes is quickly Frozen at -54 to -72°C & $H_2O$ is removed by high vaccum.

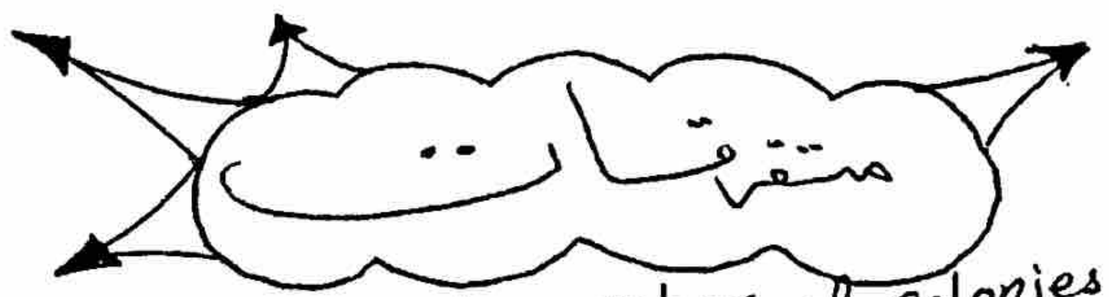
* Agar of culture media

Solid media → > 1.5% agar

semisolid media → > 0.5% agar
< 1.5% agar

Liquid (Broth) media → No agar

* E. Coli generation time = 12-15 min




- least number of colonies will be formed in quadrant #4
- usually quadrant #4 → pure culture.

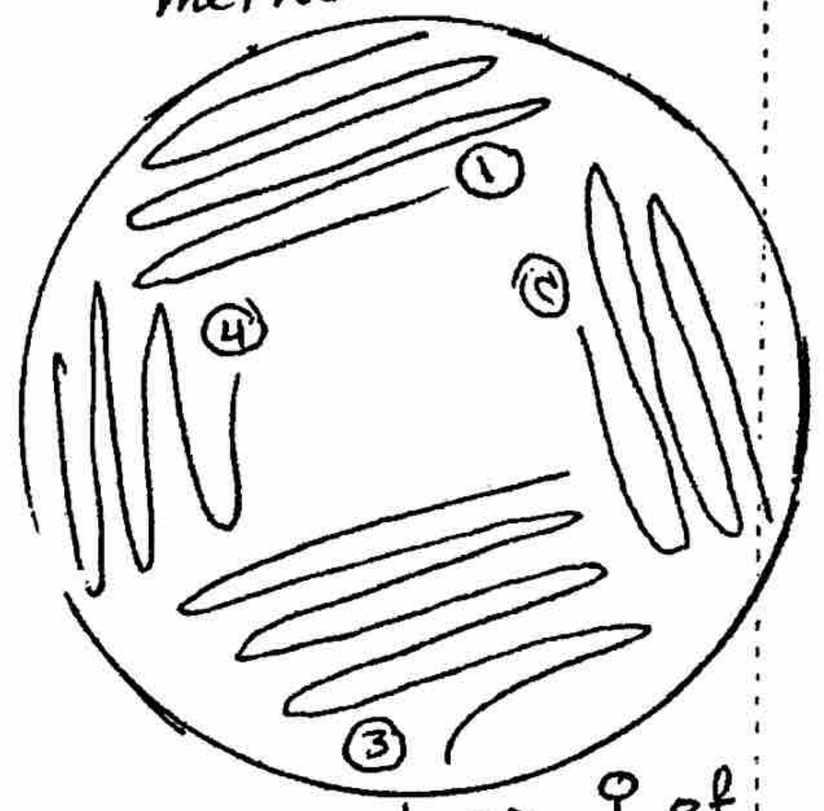
* chemostat

- environment used to continuously culturing MO
- always in Log phase
- by regulating the supply of nutrients & removal of waste products & excess MO to prevent overcrowding

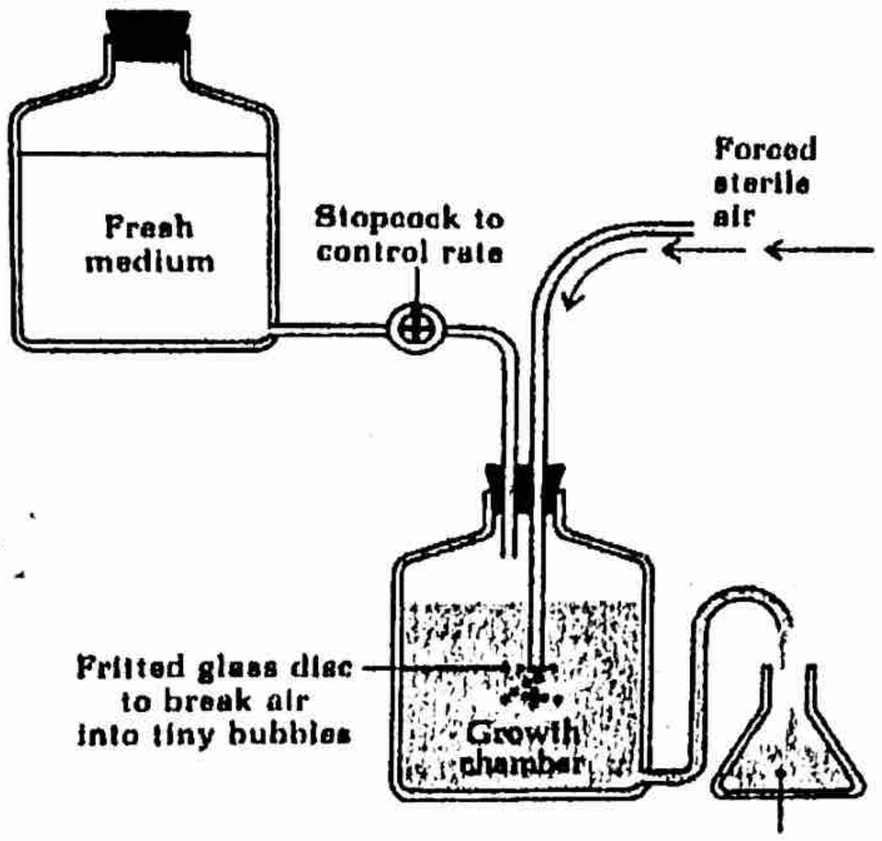
* Mixed culture = contains > 1 MO type

* pure culture = one MO type → seen in urinary cultures due to contamination

* to get pure culture we use streak plate method



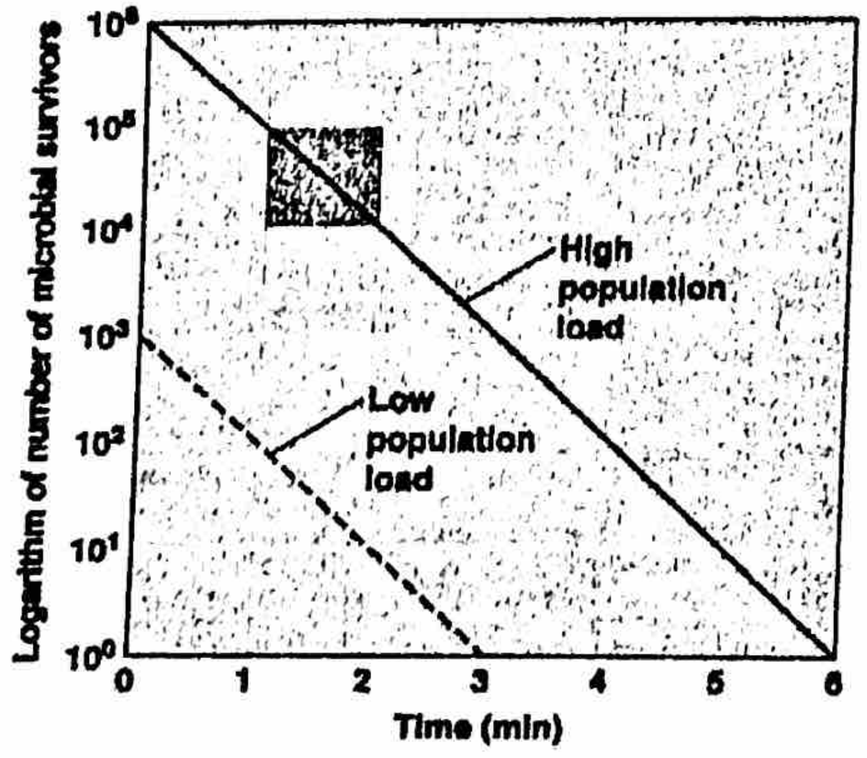

- we take a loop of the patient sample
- we divide the plate into 4 quadrants
- we make a streak (zigzag) line on the 1st quadrant
- from the 1st we take the 2nd & etc...




many industrial and research procedures depend onthe maintenance of an essential species of microorganism.

These are continuously cultured in a controlledenvironment called a chemostat, which regulatesthe supply of nutrients and the removal of waste productsand excess microorganisms.

Chemostats are used in industries where yeast is grown to produce beer and wine,where fungi and bacteria are cultivated to produce antibiotics




**Effectiveness of Antimicrobial Treatment** Depends on:

- **Number of microbes**: the more microbes, the longer it takes to eliminate the population.
- **Environmental influence**: (organic matter, temperature, biofilms) inhibit the action of chemical antimicrobials.
- **Time of exposure**: extended exposure for more resistant microbes or endospores are required.
- **Microbial characteristics**