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Factors Affecting Microbial Growth

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Microbial growth

- The microbial growth is the increase in number of cells rather than in size of individual cells.
- The requirements for microbial growth can be divided into *two* main categories:
physical and nutritional.

Factors affecting Microbial growth

→Physical factors

- pH
- Temperature
- Osmotic pressure
- Hydrostatic pressure
- Moisture
- Radiation
- Oxygen concentration

Nutritional factors

- Carbon
- Nitrogen
- Sulfur
- Phosphorus
- Trace elements
- vitamins

pH

The microorganisms are divided into different categories with respect to their pH range.

Acidophiles (0.1 – 5.4)

e.g. *Lactobacillus*

Neutrophiles (5.4 – 8.0)

e.g. *Bacterial pneumonia*

Alkaliphiles (7.0 – 11.5)

e.g. *Agrobacterium*

Temperature

Psychrophiles “cold loving bacteria” (15 -20c)

Obligate psychrophiles

These can not grow above 20c e.g. *Bacillus globisporus*

Facultative psychrophiles

It grows best below 20c but can also grow above e.g. *Xanthomonas pharmicola*

Mesophiles 25 -40c

e.g. all human pathogens

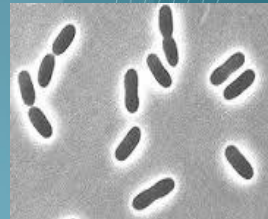
Thermophiles “Heat loving” (50 -60c)

Obligate Thermophiles

They can only grow at temperature above 37C. E.g. Archea, Eubacteria

Facultative Thermophiles

They can grow above and below 37C.



Oxygen

Obligate aerobes

Which always require oxygen to grow e.g *Pseudomonas*

Obligate anaerobes

They don't require oxygen at all for growth and respiration e.g *clostridium*

Micro aerophiles

They grow best in small amount of oxygen e.g *Campylobacter*

Aerotolerant anaerobes

They can survive in presence of oxygen but don't use it in their metabolism.

e.g *Lactobacillus*

Moisture

Unlike large organisms that have protective covering and internal fluid environments single celled are directly exposed to the environments so they need constant moisture to survive.

Osmotic pressure

Plasmolysis happens when the dissolved substances in the environment exert more pressure as compared to the substances within the cell (hyperosmotic environment) and lead to cell shrinka

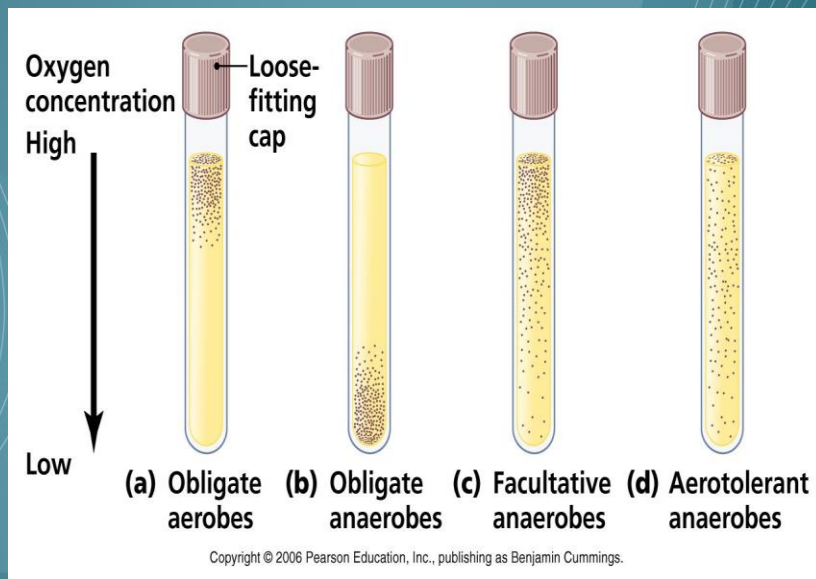
Distension when cells in distilled water have higher osmotic pressure inside as compared to the environment and gain water and cell become turgid to prevent bursting. This is called distension.

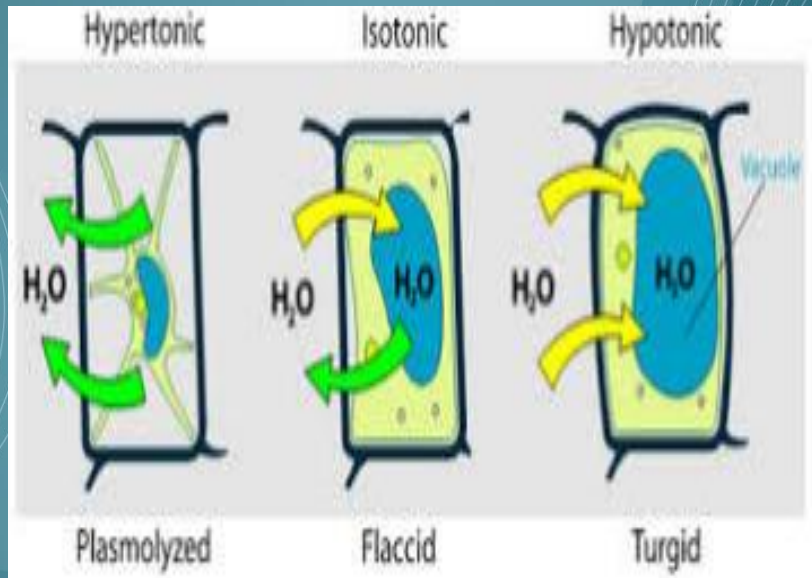
Radiation

Radiations like U V rays and gamma rays can mutate DNA and kill microorganisms, the bacterium *Deinococcus radiodurans* can survive harsh radiations such microbes are used in cleaning up the contaminated sites.

Hydrostatic pressure

Many microbes have to face water pressure specially in lakes and oceans and those who survive in such habitat are **Barophiles**.





Nutrition

Carbon sources:

- Carbon is used as energy source.
- Its compounds as building blocks to synthesize cell components

•Nitrogen sources:

- Microbes need nitrogen to synthesize enzymes, other proteins and nucleic acids.
- Some obtain by inorganic sources.
- They reduce nitrate ions to amino groups and use amino groups to make amino acids then these are used in protein synthesis.

•Sulfur and phosphorus:

- Microbes obtain sulfur from inorganic sulfate salts and sulfur containing amino acids.
- They use them to make proteins, coenzymes and other cell components.
- Microbes obtain phosphorus mainly from inorganic phosphate ions.
- They use phosphorus to synthesize ATP phospholipids and nucleic acids.

Trace elements

- Microbes require trace elements copper, iron, zinc, and cobalt usually in the form of ions.
- Trace elements serve as cofactors in enzymatic reactions potassium,
- zinc magnesium and manganese are used to activate certain enzymes.
- cobalt is required to synthesize vitamin B12
- Iron is required for the synthesis of heme containing compounds .
- Calcium is required by gram positive bacteria for the synthesis of cell wall and by spore forming organisms for the synthesis of spores.

Vitamins

- . An organic compound that an organism requires in small amount and used as a coenzyme.
- . These include folic acid, vitamin B12, and vitamin K. Human pathogens require the vitamins and then they become able to grow in the host by receiving these from the host.
- . Microbes living in human intestine manufacture vitamin K which is necessary for blood coagulation.

Environmental perspective

- Environment is rich reservoir for the growth of microbes specially soil in which all essential elements that are required for the growth of microbes are present.
- Environment contains all types of habitats to support different categories of microbes starting from pH, oxygen, temperature and pressures.
- This mechanism is maintained by the biological processes of which the microbes are a part, for example nitrogen and carbon cycling.
- By isolating the microbes from environment and knowing the composition of their habitat, now they are cultured in the laboratories by providing with all



Culture Media

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Culture media

A nutrient material prepared for the growth of microorganisms in a laboratory is called a culture medium.

- A wide variety of media are available for the growth of microorganisms in the laboratory.
- Media are constantly being developed or revised for use in the isolation and identification of bacteria that are of interest to researchers in such fields as food, water, and clinical microbiology.



- Microbes that are introduced into a culture medium to initiate growth are called an **inoculum**.
- The microbes that grow and multiply in or on a culture medium are referred to as the **culture**.
- **sterile**-that is, it must initially contain no living microorganisms-so that the culture will contain only the microbe (and their offspring) we add to medium.



Agar

- a solidifying agent added to the culture medium.

- A complex polysaccharide derived from a marine alga.

It is generally not degraded by microbes

- Liquefies at 100°C
- Solidifies at $\sim 40^{\circ}\text{C}$



Classification Of Media

1. Consistency
2. Nutritional
3. Functional

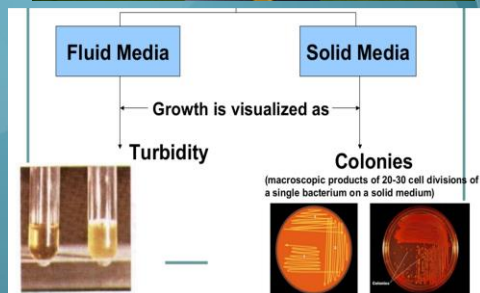
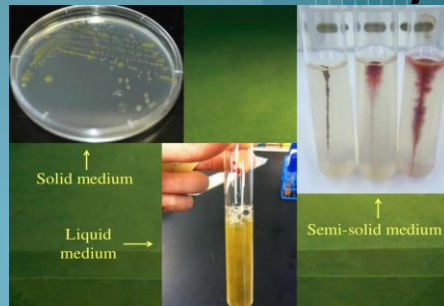
Classification based on Consistency

- Based on agar concentration:

1.Solid media: 2% agar
e.g. Nutrient agar

2.Liquid media:
Absence of agar e.g.
nutrient broth

3.Semi solid media:
0.2-0.5% agar e.g.
peptone water



Chemically defined medium

- A **chemically defined medium** is one whose **exact chemical** composition is known.
- For a chemoheterotroph, the chemically defined medium must contain organic growth factors that serve as a source of carbon and energy.

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 ($\text{NH}_4\text{H}_2\text{PO}_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

Classification based on Nutritional compotents

- 1.Simple media
- 2.Complex media
- 3.Synthetic media



Classification based on Functional

- Selective medium
- Differential medium
- Enrichment medium
- Reducing medium

Selective medium

- It is designed to inhibit the growth of undesired organisms and to encourage the growth of the desired microbes.
- Various methods to make it selective include: Addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these medium.
- For example, **bismuth sulfite agar** is one medium used to isolate the typhoid bacterium, the gram-negative *Salmonella typhi* from feces. *Bismuth sulfite inhibits gram -positive bacteria* and most gram-negative intestinal bacteria (other than *S. typhi*), as well.
- **Sabouraud's dextrose agar**, which has a pH of 5.6, is used to isolate fungi that outgrow most bacteria at this pH.

Differential medium

- It distinguishes one microorganism type from another based on a difference in colony appearance (color, shape or growth pattern) on the media.

Bacterial colonies on differential media have a distinctive appearance. This medium is **mannitol salt agar**, and the bacteria in the colonies capable of fermenting the mannitol in the medium to acid, cause a change of color. Actually, this medium is also selective because the high salt concentration prevents the growth of most bacteria except *Staphylococcus* spp



Reducing medium

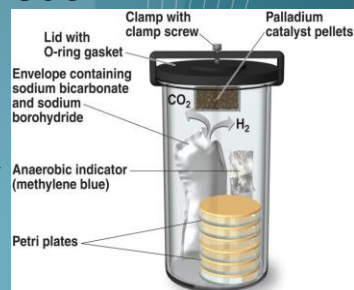
- These media contain ingredients, such as sodium thioglycolate, that chemically combine with dissolved oxygen and deplete the oxygen in the culture medium.
- The cultivation of anaerobic bacteria poses a special problem. Because anaerobes might be killed by exposure to oxygen, special media called **reducing media must be used.**

Anaerobic growth methods



Figure 6-22 Microbiology 4/e
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To culture obligate anaerobes, all molecular oxygen must be removed and kept out of medium. Agar plates are incubated in sealed jars containing chemical substances that remove oxygen and generate carbon dioxide or water



An anaerobic chamber. The technician is pipetting a bacterial suspension into a flask inside an anaerobic chamber filled with an inert oxygen-free gas. His arms and hands are encased in glove ports. Organisms and materials enter and leave through the air-lock opening that is visible to the left.



Importance Of Culture Mediums

- As large number of population of bacteria in the nature so
By appropriate procedures they have to be grown separately (isolated) on culture media and obtained as pure culture for study, subsequent clinical diagnoses.
- Bacteria have to be cultured in order to obtain antigens from developing serological assay for vaccines.
- Certain genetic studies and manipulations of the cells also need that bacteria to be cultured in vitro.

Phases of Growth

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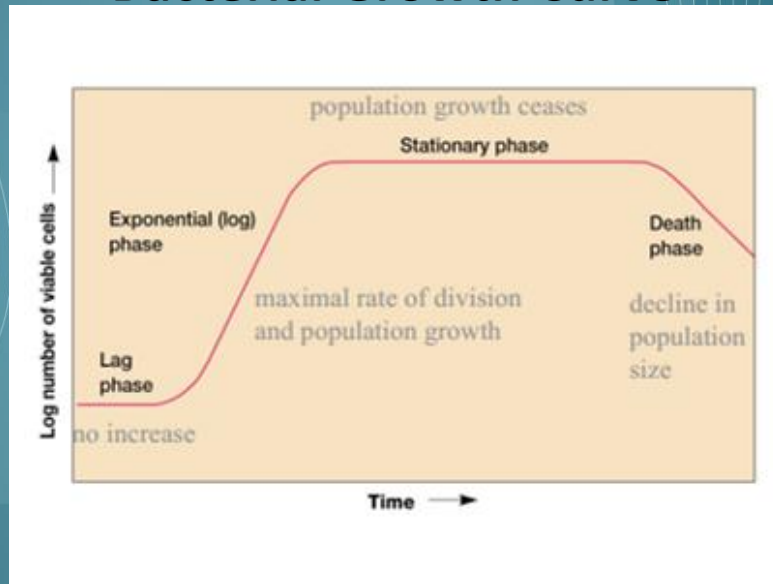
GROWTH

- It is defined as an increase in cellular constituents that may result in either
 - Increase in cell number; or Increase in cell size
 - However when it comes to microorganisms, microbiologists usually study population growth rather than growth of individual cells

BACTERIAL GROWTH CURVE

- Microorganisms are cultivated in batch culture
- Incubated in a closed vessel with a single batch of medium
- Plotted as logarithm of cell number versus time
- Growth of bacterial populations follow a sequential series of four distinct growth phases;
- The Lag, Log, Stationary and Death phases.

Bacterial Growth Curve



LAG PHASE

- When introduced into fresh medium, bacterial cells from dormant state become actively growing state
- Cells synthesize new components or enzymes to replenish spent materials or to adapt to the new medium or conditions
- Phase varies in length from less than an hour to many days depending on the species

EXPONENTIAL/LOG PHASE

- Bacteria actively undergoing binary fission
- If conditions of sufficient nutrients and negligible waste accumulation persists, rate of growth is exponential and constant
- Bacterial population is most uniform in terms of chemical and physical properties during this phase
- Since the number of bacteria increase rapidly every generation, logarithmic graph is used for the representation of bacterial growth
- Most of the environmental and microbial research is done on bacteria in this phase

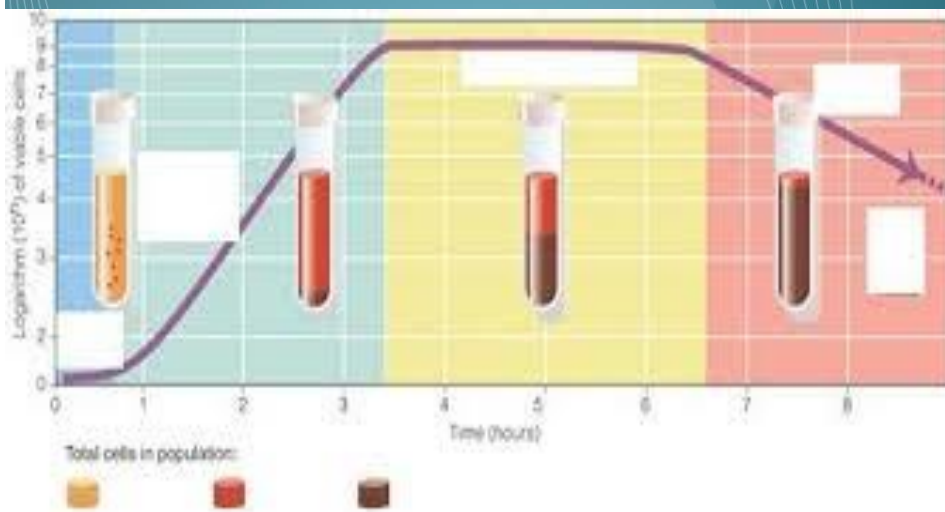
GENERATION TIME

- Bacteria double their number every generation time
- Generation time varies from species to species generally from 20 minutes to a few days.
- Growth can be calculated
 - $N_t = N_0 \times 2^n$
 - (N_t) number of cells in population
 - (N_0) original number of cells in the population
 - (n) number of divisions
- Growth rate (min⁻¹) = 1 / Mean Generation Time

STATIONARY PHASE

- Once cells have grown for a while, the nutrients begin to diminish.
- Cells may begin to die.
- This may be because metabolically active cells stop reproducing, and are balanced by the dying cells within the medium.

Bacterial Cells in four phases



POSSIBLE REASONS FOR ENTRY INTO STATIONARY PHASE

- **Nutrient Limitation**
- **Limited Oxygen Availability**
- **Toxic Waste Accumulation**
- **Critical Population Density reached**

STARVATION RESPONSES

- **Morphological changes e.g. Endospore formation**
- **Decrease in cell size, protoplast shrinkage, and nucleoid condensation**
- **Production of Starvation Proteins**
- **Long term Survival**
- **Increased Virulence**

DEATH PHASE

- Extensive depletion of nutrients and waste accumulation
- Cells dying at exponential rate
- Irreversible loss of ability to reproduce
- In some cases, death rate slows due to accumulation of resistant cells
- If cells are to survive, they have to switch from metabolically active state into dormant state because there are no available nutrients for cell's growth

