

2nd year Medical Students - JU

Bacterial growth, physiology & metabolism

Dr. Hamed Al Zoubi

Associate Professor of Medical Microbiology.

MBBS / J.U.S.T

MSc, PhD/ UK

Bacterial physiology, metabolism and growth

- Contents:

1. Growth

2. Metabolism & physiology.

3. Summary.

Bacterial growth, physiology and metabolism

Bacterial survival = growth & replication.

Can not just sit around.

*Growth stages include metabolism, regulation & division.

- Fast growing bacteria that divide each 10-30 minutes e.g *Vibrio* .
- Slow growing: each 24 hours e.g *Mycobacterium tuberculosis*.
- Bacteria consists of many structures & elements e.g protein, polysaccharides, lipids, nucleic acid & peptidoglycan.
- Growth needs materials (nutrient) & energy/metabolism.

Bacterial growth

- Increase in the size of organisms and an increase in their number.
- Whatever the balance between these two processes, the net effect is an increase in the total mass(*biomass*) of the culture.
- The number will be adopted here, as outcome of infections and in the measurement of the effects of antibiotics.

Types of growth in the laboratory

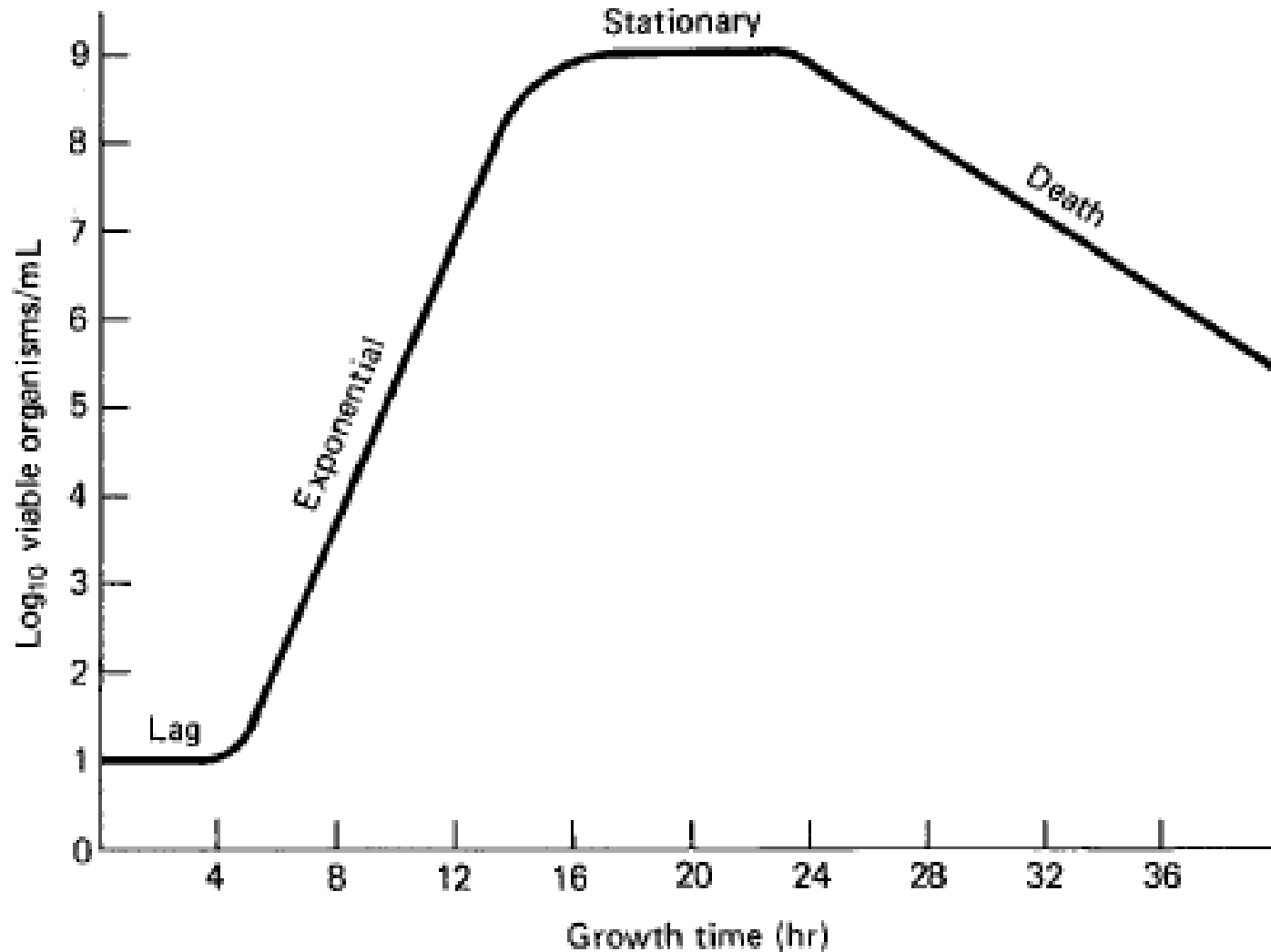
- In the laboratory, bacterial growth can be seen in three main forms:
- 1. By the development of *colonies, the macroscopic product of 20–30 cell divisions of a single cell.*
- 2. By the transformation of a clear broth medium to a turbid suspension of 10^7 – 10^9 cells per mL.
- 3. In *biofilm formation, in which growth is spread thinly (300–400 μm thick) over an inert surface and nutrition obtained from a bathing fluid.*

- A biofilm is a layer of prokaryotic organisms that have aggregated to form a colony. The colony attaches to a surface with a slime layer which aids in protecting the microorganisms. Biofilms often form on the inert surfaces of implanted devices such as catheters, prosthetic, cardiac valves and intrauterine devices.

Bacterial growth stages

1. **Lag phase**, there is little or no change in the number of cells (adjustment stage), but metabolic activity is high.
2. **Log or exponential phase**, the bacteria multiply at the fastest rate possible under the conditions provided. The bacterial population doubling occurs at a constant rate.
3. **Stationary phase**, there is an equilibrium between cell division and death (nutrients start to deplete & toxic materials start to be produced).
4. **Death (decline) phase**, the number of deaths exceeds the number of new cells formed.

Growth curve/growth phases in liquid medium



Extending log phase

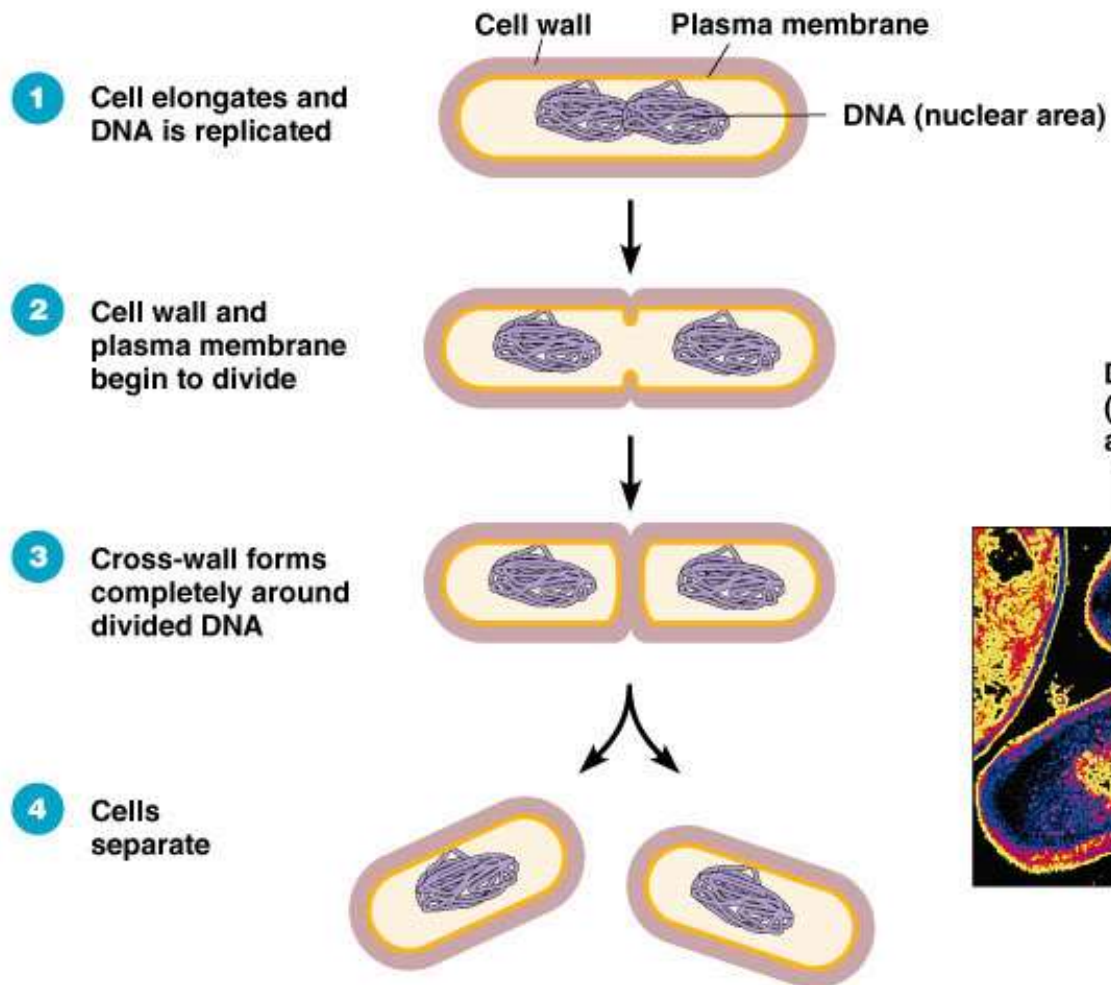
- Chemostat(chemical environment is static) : cells of a growing culture are harvested continuously and nutrients replenished continuously
- Maintenance of bacteria in continuous culture is sometimes necessary in industrial and research purposes

Media to isolate bacteria

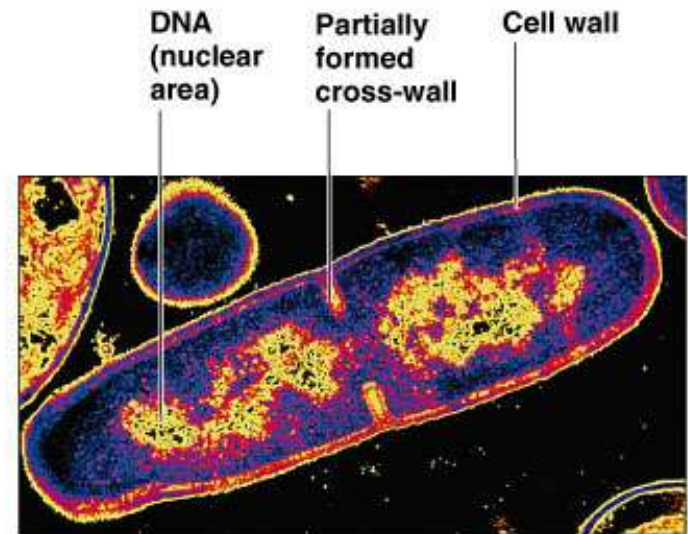
- Main features of media in medical bacteriology are:
 - 1. a source of protein or protein hydrolysate, often derived from casein.
 - 2. control of pH in the final product (after sterilization)
 - 3. a defined salt content

Bacterial division and generation time

1. The reproduction method of bacteria is binary fission, in which a single cell divides into two identical cells. Some organisms reproduce by budding, aerial spore formation or fragmentation.
 - Cell division occurs by the development of constrictions mediated by the assembly of an actin-like protein.
 - constrictions proceed from the periphery inwards and, in some cases, produce a transverse cell wall known as a *septum* or *cross-wall*



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



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

In the laboratory

- **Culture media** is a nutrient material prepared for the growth of bacteria in a laboratory.
- Microbes that grow and multiply in or on a culture medium are known as a culture.
- **Agar** is a common solidifying agent for a culture medium.
- Agar media are usually contained in Petri dishes or test tubes (slant or deep)

In the laboratory



In the laboratory

- Growth requirements in the lab.:
 1. **Fastidious** organisms require many nutrients.
 2. **Simple** requirements can make everything from scratch.
 3. selective (enrichment) with indicator.
 4. Some bacteria **cannot be cultured *in vitro* (Lab.)**.
 - a. *Chlamydia* and *Rickettsia* : need **tissue culture** like viruses
 - b. *Treponema pallidum*, *Mycobacterium leprae*, require animal infection.
 5. Cannot predict virulence by growth (some slow or non-culturable bacteria can be fatal).

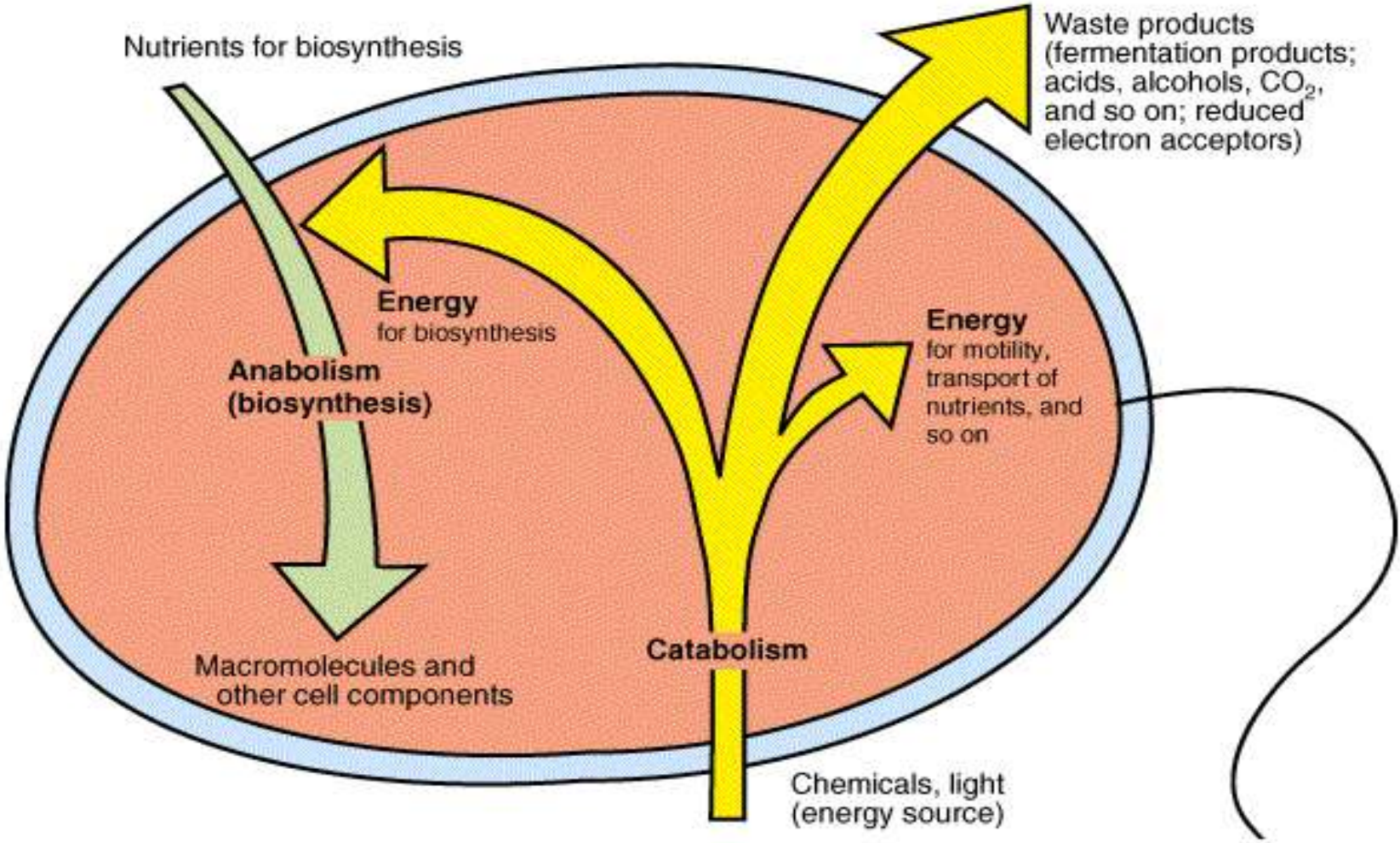
Bacterial physiology and metabolism

- Metabolism in bacteria leads to faster growth than our bodies metabolism.
- Bacteria use many compounds as energy sources.
- Bacterial nutritional requirements much more diverse than our cells requirement.
- Some biosynthetic processes, such as those producing lipopolysaccharide (LPS), and teichoic acid, are unique to bacteria.

Bacterial physiology and metabolism

- * Bacterial metabolism involves approx. 2000 chemical reactions. These reactions can be categorised according to their function in the metabolic processes of **fueling, biosynthesis, polymerization and assembly.**
- Energy Production = Energy Consumption.
- Metabolism = Anabolism + Catabolism.
- Anabolism = synthesis.
Catabolism = degradation.
- Understanding physiology & metabolism is necessary for bacterial identification & to design antibacterial agents.

Bacterial physiology and metabolism



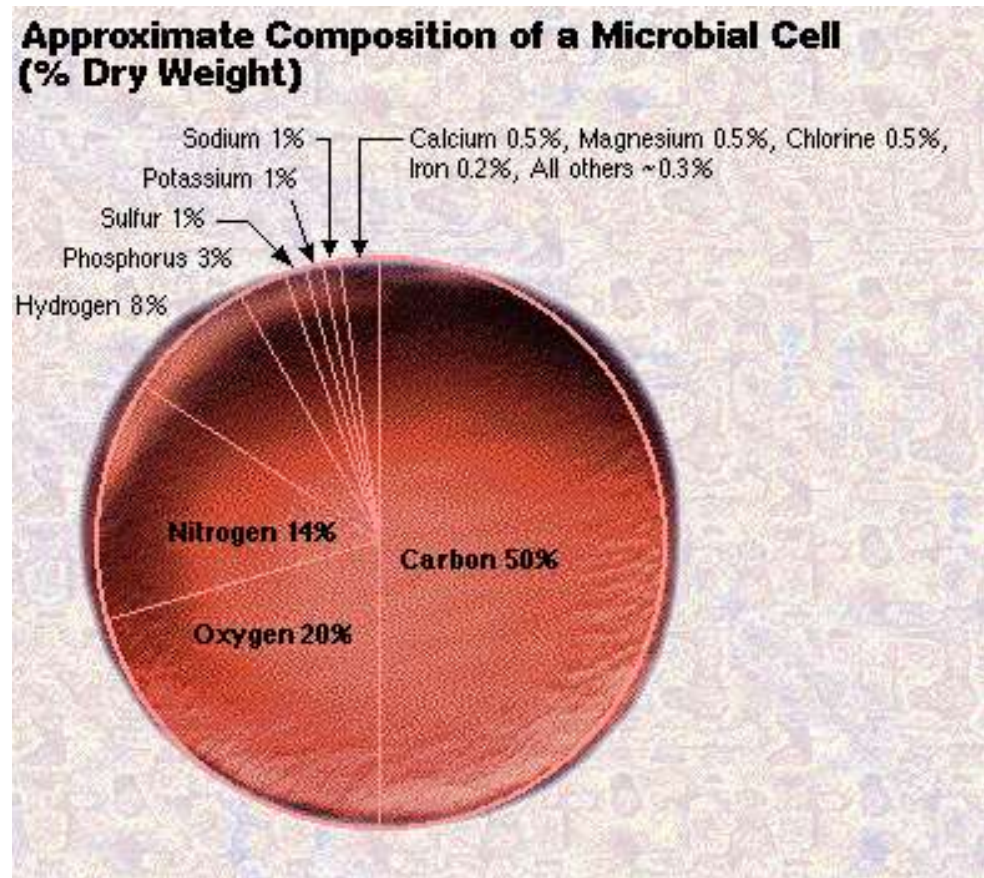
Bacterial physiology and metabolism

Nutritional requirements:

1. includes many elements like:
 - A. carbon, hydrogen, O₂, nitrogen, phosphorus & sulphur: needed for the synthesis of structural components.
 - B. potassium, calcium magnesium and iron: needed for cellular functions.
2. Can be obtained from simple elements or by breaking down large molecules such as protein breakdown into amino acids using bacterial enzymes.

Bacterial physiology and metabolism

- many bacteria have to synthesize some nutrients such as folic acid which makes these bacteria susceptible to agents that interfere with the biosynthesis of folic acid, e.g by trimethoprim & sulfonamides antibiotics.



Bacterial physiology and metabolism

- Nutritional requirements differ among bacteria and can be used for identification.
- Nutrients can be obtained from different sources.
 1. Elements such as:
 - A. hydrogen & oxygen are obtained from water.
 - B. Carbon: usually obtained from degradation of carbohydrates by **oxidation** or **fermentation**.

Carbon is necessary to provide energy in the form of ATP (adenosine triphosphate).
 - C. Nitrogen: from ammonia in the environment or proteins '**deamination**' using bacterial enzymes.

Bacterial physiology and metabolism

2. Organic factors (from exogenous source/can't be synthesized by bacteria) such as:

Amino acids: e.g from proteins breakdown.

Purines and pyrimidines:

- * Nucleic acid precursors.

- * must be converted into nucleotides(sugar+base+phosphate) & nucleosides(sugar+base) before being incorporated into the **DNA** or **RNA**.

3. Vitamins: most are needed for the formation of coenzymes in some bacteria.

Major elements sources

Major elements, their sources and functions in bacterial cells.

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO ₂	Main constituent of cellular material
Oxygen	20	H ₂ O, organic compounds, CO ₂ , and O ₂	Constituent of cell material and cell water; O ₂ is electron acceptor in aerobic respiration
Nitrogen	14	NH ₃ , NO ₃ , organic compounds, N ₂	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H ₂ O, organic compounds, H ₂	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO ₄)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO ₄ , H ₂ S, S ₀ , organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

ENERGY SOURCE

- * **Phototrophs**: use light as their energy
- * **Chemotrophs**: obtain energy from the oxidation of chemical compounds (either organic or inorganic)

Hydrogen source

- ***organotrophs** : extract electron or hydrogen from organic compounds
- ***lithotroph**: if it can use inorganic sources (e.g. ammonia or hydrogen sulphide), use reduced inorganic substances

- Energy and Hydrogen donor designations are referred to routinely by combining the two terms:
 - *Chemo-organotrophs*
 - (the vast majority of currently recognized medically important organisms)
 - *chemolithotrophs e.g. some Pseudomonas spp.*

CARBON SOURCE

- a. **Autotrophs** —can draw carbon from carbon dioxide

- b. **Heterotrophs** —carbon from organic compounds

- c. **Mixotrophic** - carbon is obtained from both organic compounds and by fixing carbon dioxide

These requirements can be combined:

- *Energy and carbon sometimes:*
- **Chemoheterotrophs** —energy from chemical compounds, carbon from organic compounds, this group includes most as well as all protozoa, fungi, and animals.

Bacterial physiology and metabolism

- Inside the cell, sugar molecules or other sources of carbon and energy are metabolized by different pathways, mainly by:
 1. the Embden–Meyerhof glycolytic pathway.
 2. the pentose phosphate pathway.
 3. the Krebs cycle to yield the carbon compounds needed for biosynthesis.
- Bacteria generate energy by two ways fermentation and / or oxidation.

Bacterial physiology and metabolism

- COMPARISON OF METABOLISM:

1. Aerobic respiration (oxidation):

- * Total ATP Prokaryotes=38, Eukaryotes=34

- Final electron receptor is usually oxygen.

2. Fermentation:

- * Yield = 2 ATP (less efficient).

- * Final electron receptor is organic molecule.

- * End products: acids/Alcohol.

CO₂ is produced in both.

Bacterial physiology and metabolism

Environmental conditions governing growth:

1. Temperature: majority of medically important bacteria are mesophiles, grow at 37°C (i.e body temperature):

- 2 . water: at least 80% of bacterial cell consists of water

Bacterial physiology and metabolism

3 . Oxygen and Carbon dioxide.

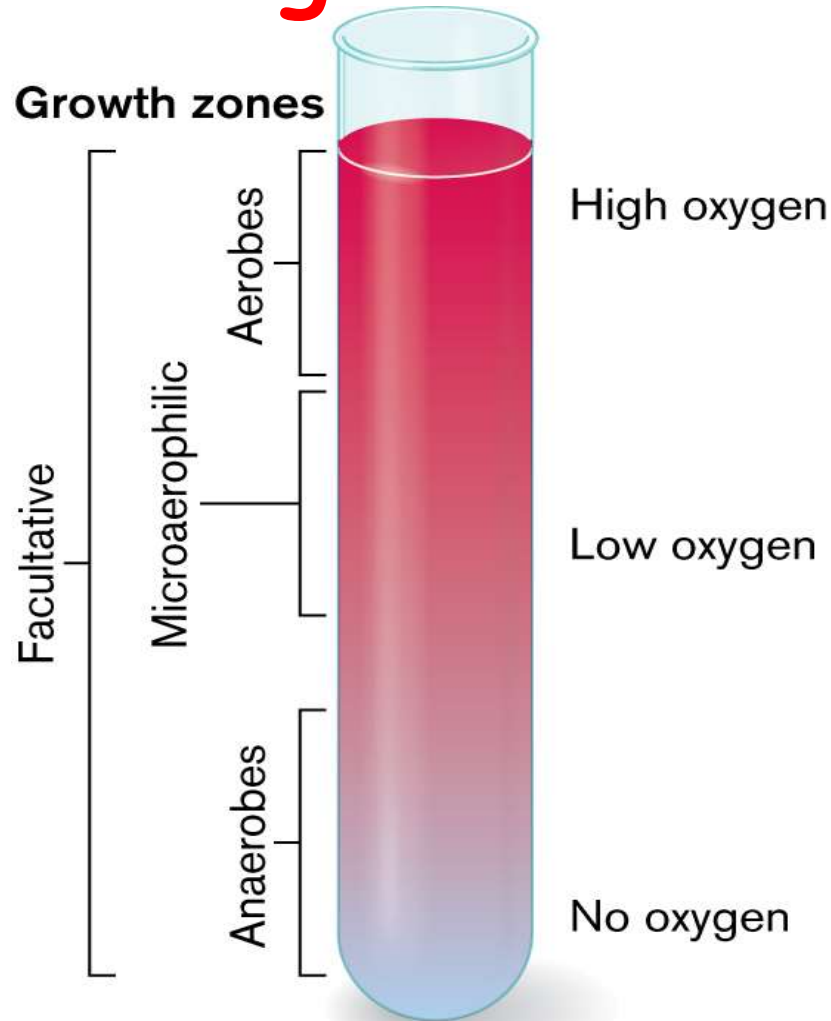
- * Metabolism in the presence of oxygen may give rise to some toxic substances, **hydrogen peroxide (H_2O_2)** and the **superoxide anion (O^{2-})**.
- * Superoxide is partially detoxified by an enzyme, **superoxide dismutase**, Hydrogen peroxide is degraded by **peroxidases (Catalase)**.
- Bacteria that possess these protective enzymes can grow in the presence of oxygen.
- In response to oxygen, bacteria can be placed in five categories as in the next Figure:

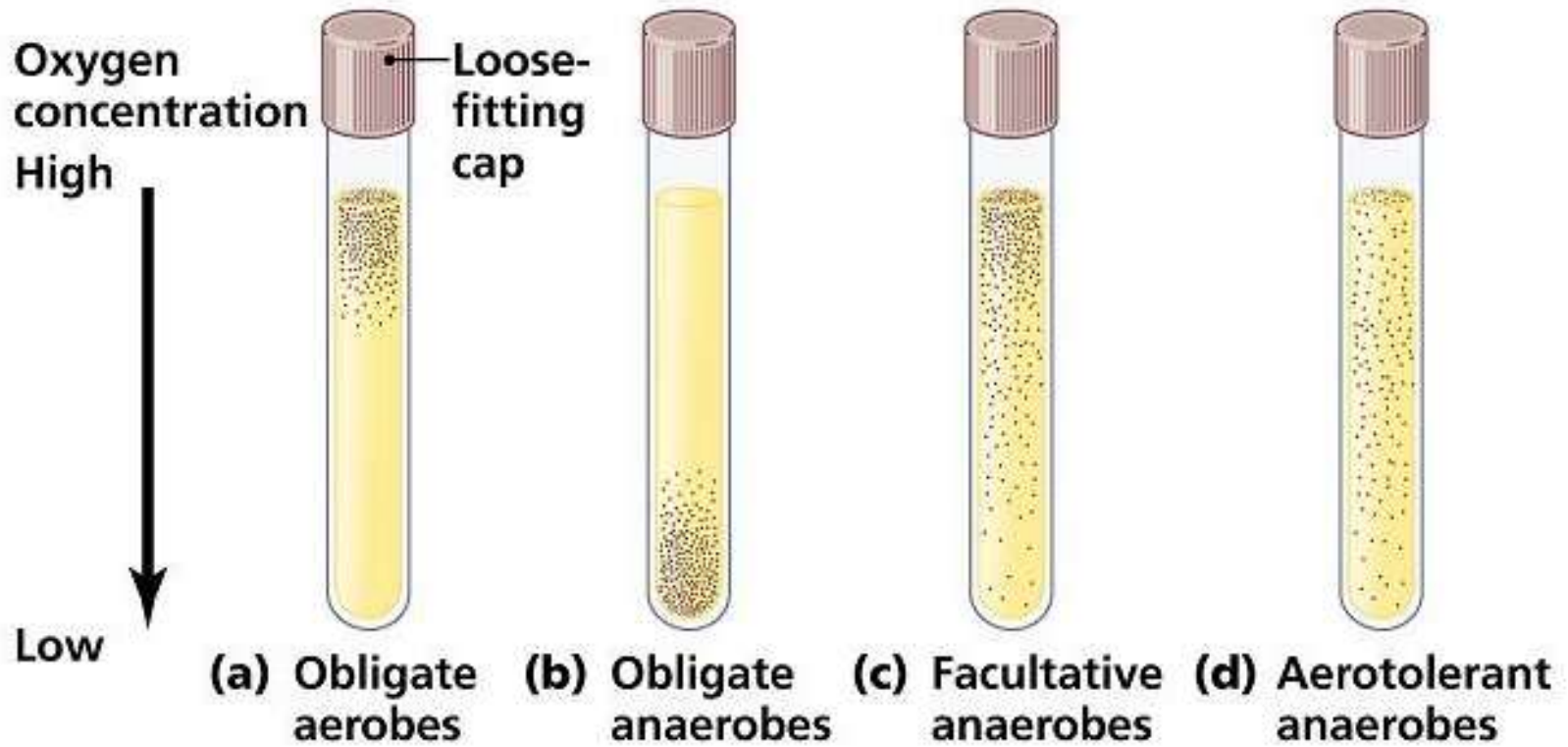
Table 4.1 Key descriptive terms used to categorize bacteria according to their growth requirements

Descriptive term	Property	Example
Growth atmosphere		
Strict (obligate) aerobe	Requires atmospheric oxygen for growth	<i>Pseudomonas aeruginosa</i>
Strict (obligate) anaerobe	Will not tolerate oxygen	<i>Bacteroides fragilis</i>
Facultative anaerobe	Grows best aerobically, but can grow anaerobically	<i>Staphylococcus</i> spp., <i>E. coli</i> , etc.
Aerotolerant anaerobe	Anaerobic, but tolerates exposure to oxygen	<i>Clostridium perfringens</i>
Micro-aerophilic organism	Requires or prefers reduced oxygen levels	<i>Campylobacter</i> spp., <i>Helicobacter</i> spp.
Capnophilic organism	Requires or prefers increased carbon dioxide levels	<i>Neisseria</i> spp.
Growth temperature		
Psychrophile	Grows best at low temperature (e.g. <math><10^{\circ}\text{C}</math>)	<i>Flavobacterium</i> spp.
Thermophile	Grows best at high temperature (e.g. >math>>60^{\circ}\text{C}</math>)	<i>Bacillus stearothermophilus</i> ^a
Mesophile	Grows best between 20–40°C	Most bacterial pathogens

^a Not a pathogen; its spores are very heat resistant and are used for testing the efficiency of heat sterilization.

Oxygen-related growth zones in a standing test tube





pH

- Majority of bacteria grow **BEST** at neutral or slightly alkaline pH
 - pH 7.0 – 7.4 => this is near most normal body fluids ?
- **Acidophiles**: grow **BEST** at low pH (acid: pH 0 – 1.0)
 - T.B. - pH 6.5-6.8
- **Alkalophiles**: grow **BEST** at high pH (alkaline: pH 10.0)
 - *V. cholerae* - pH 8.4-9.2,

Bacterial physiology and metabolism

Summary

- Bacterial growth is an active mechanism.
- Bacteria has different nutritional needs and nutritional uptake mechanisms.
- Nutrients are metabolised using many bacterial pathways.
- Growth has many phases and it is affected by the surrounding environment e.g oxygen & temperature.
- Bacteria has to replicate its DNA in order to pass it to the offspring.

Bacterial growth, physiology and metabolism

The End