Microbial growth and metabolism

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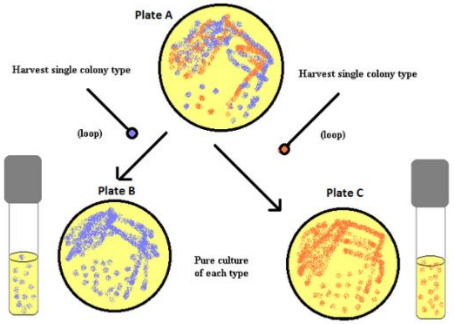
Microbial growth and microbial growth requirement

Microbial growth

Microbial growth is the increase in number of cells, not cell size.

Growth in colonies

- A pure culture contains only one species or strain.
- A colony is a population of cells arising from a single cell or spore or from a group of attached cells.
- a unit used to estimate the number of viable microbial cells is colony forming unit (CFU)



Bacterial Division

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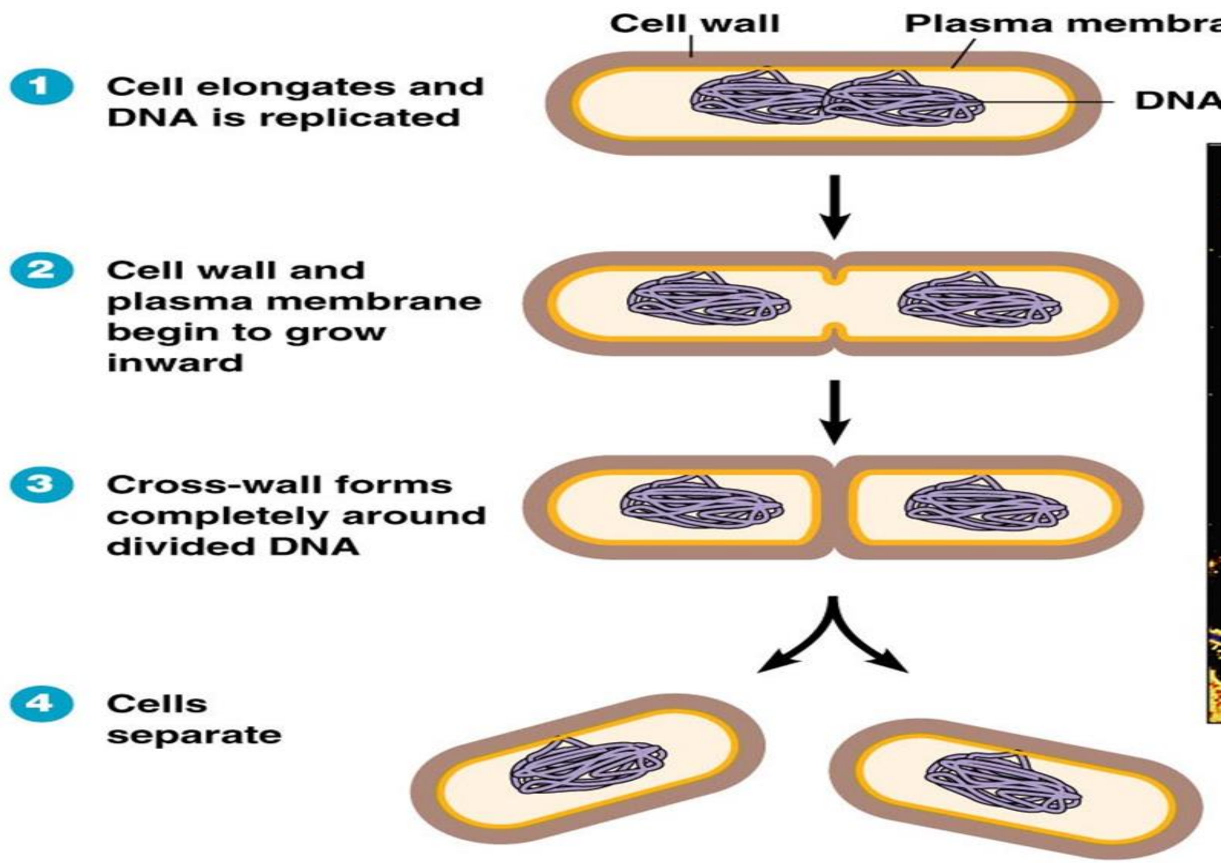


(a) A diagram of the sequence of cell division.

Growth rate

The number of generation per hour.

Generation Time

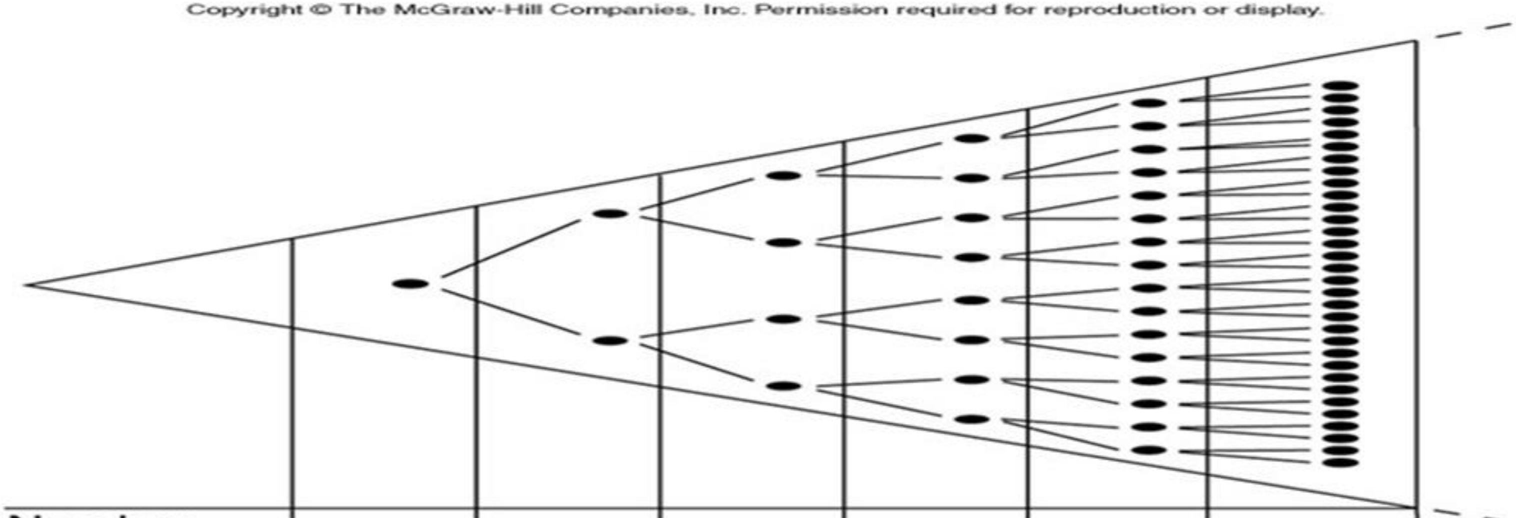
The **time required for** a cell to divide or a **population to double** is known as the generation time.

Escherichia coli	10 minutes
Mycobacterium tuberculosis	15 hours

<u>The generation time depend upon</u>:

1- The nutrient in the medium.

2- Physical condition (pH, temp. etc.)

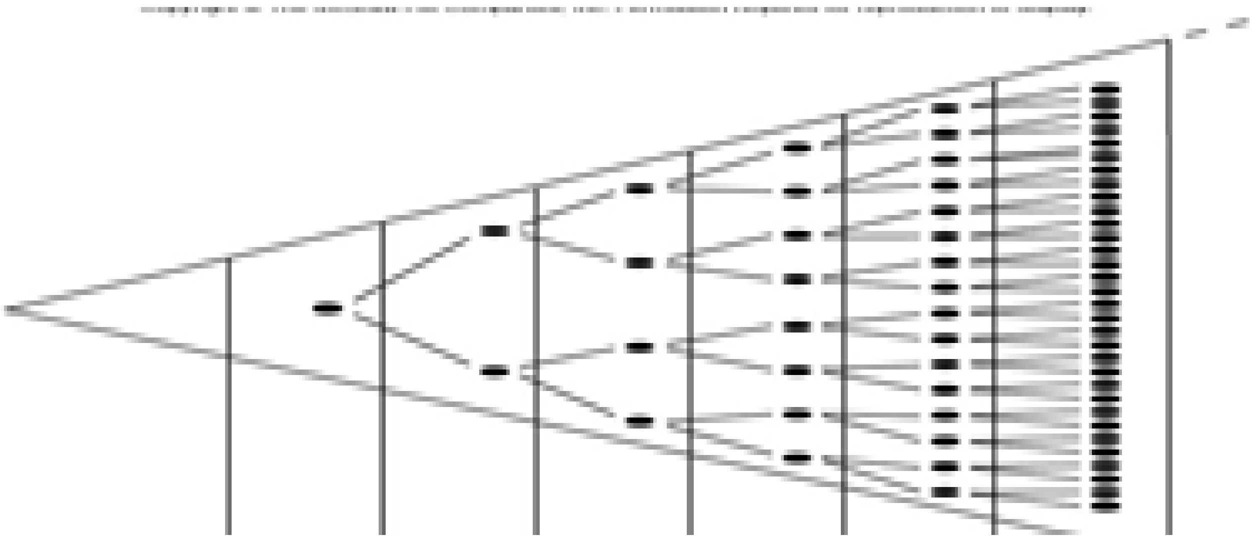


Number of cells	1	2	4	8	16	32
Number of generations		1	2	3	4	5
Exponential value		2^1 (2×1)	2^2 (2×2)	2^3 (2×2×2)	2^4 (2×2×2×2)	2^5 (2×2×2×2×2)

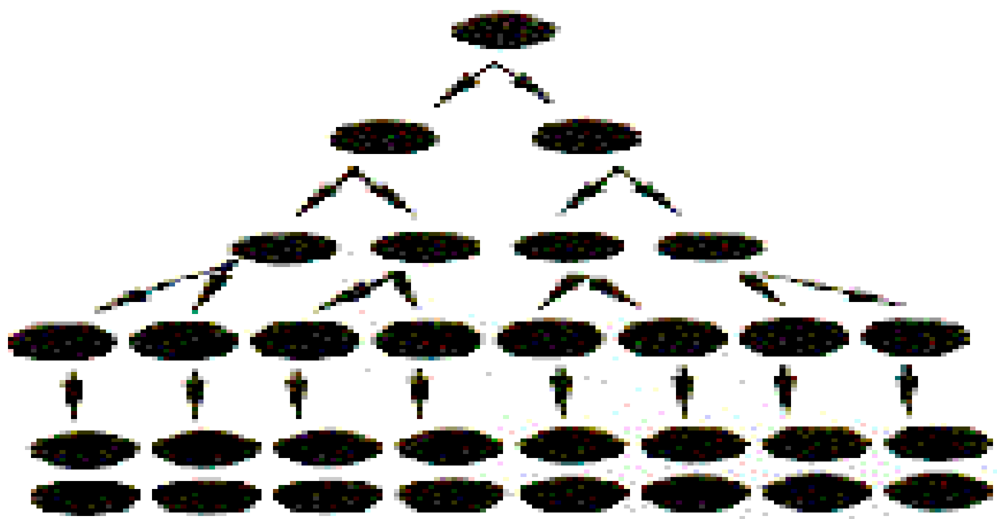
(a)

Q/- what is the generation number of bacteria when the number of cell is 8

- If the generation number of bacteria was 4 what is the Exponential value?



Mathematical Expression of Growth



Number of Generations	Cell Numbers
0	1 or 2^0
1	2 or 2^1
2	4 or 2^2
3	8 or 2^3
4	16 or 2^4
n	N or 2^n

Bacterial growth curve

- All microorganisms undergo similar growth patterns

Each growth curve has 4 phases:

1- Lag phase

2- Log phase

3- Stationary phase

4- Death or decline phase

Between each phases there is a transitional phase is represent the time require by all the cell before get to inter the new phase.

1- Lag phase:

- The number of the population remains constant.

- Microorganism start to adapted itself to the environment.

- The lag phase is generally longer if the cells are taken from an old or refrigerated culture.
- If the cells are taken from young, new growing culture (microbial population) and inoculated to a fresh medium the lag phase may be short or even absent

2- Log phase (logarithmic phase or exponential phase):

- The bacteria multiply at the fastest rate possible under the conditions provided.
- Most research is performed on cells during log phase

- This phase is called log phase because the logarithm of the bacterial mass increases linearly with time,
- and exponential growth phase because the number of cells increases as an exponential function of 2n (i.e. 2^1 , 2^2 , 2^3, 2^4 ,2^5 and so on).

- The portion of the growth curve where rapid growth of bacteria is observed is known as Logarithmic phase.

3- Stationary phase:

- Growth levels off.
- Cells per volume does not increase or decrease.
- Growth rate = Death rate.

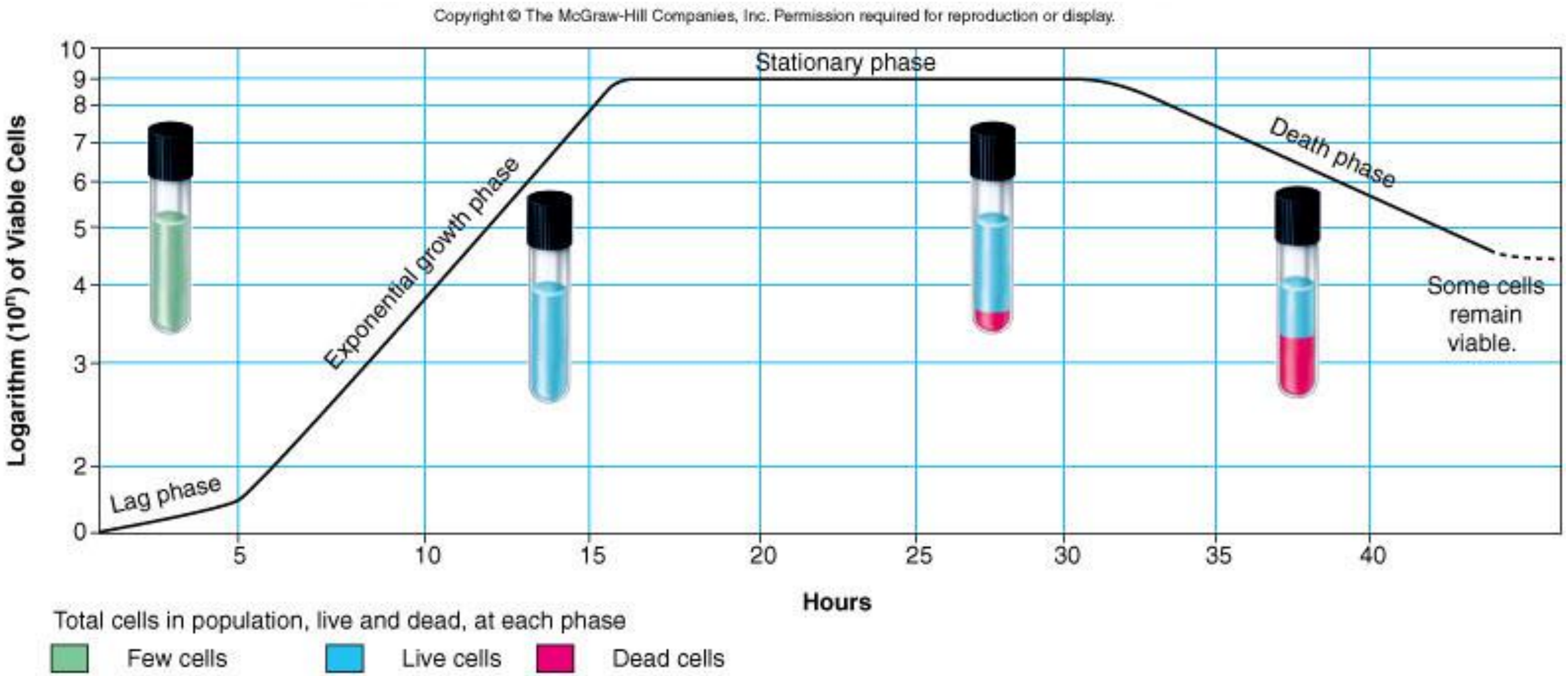
Due to

- Depletion of nutrients
- Increase in waste products

4- Death phase:

- The number of deaths exceeds the number of new cells formed
- Cells per volume decreases
- Due to
  - Very low concentrations of nutrients
  - Very high concentrations of waste products

Standard Growth Curve



Nutritional Requirements for Microorganisms:

-Water (preservation of a microbial culture from drying)

-Energy:

Phototroph- Energy from sun light (cyanobacteria)

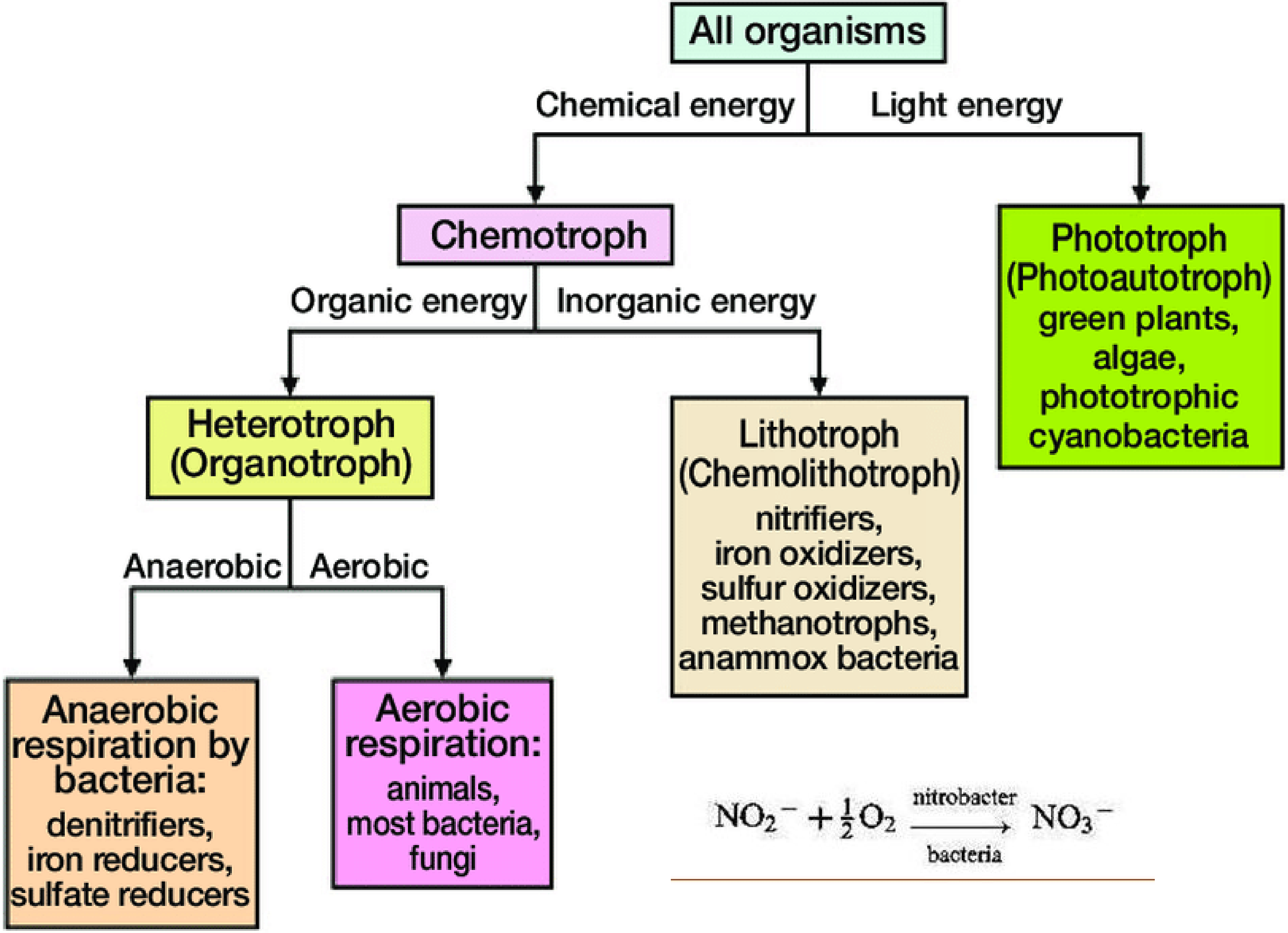
Chemotroph- Energy from chemicals (Nitrobacter, **Sulphur bacteria)**

Heterotroph- from carbohydrate and other organic material

-Carbon:

- Autotrophs- carbon from carbon dioxide- (inorganic carbon)
- Heterotroph- carbon from organic carbon

- e.g. Carbohydrates, lipids, protein.



$$NO_2^- + \frac{1}{2}O_2 \xrightarrow[\text{bacteria}]{\text{nitrobacter}} NO_3^-$$

- Essential elements

- Hydrogen (H), Sulfur (S), Oxygen (O), Phosphorous (P)
- Nitrogen (N)

 - commonly supplied as ammonia (NH4)
 - some microbes fix atmospheric nitrogen (N2)

- Trace elements

 - Required in small amounts
 - Copper (Cu), Zinc (Zn), Selenium (Se)

Environmental requirements for growth

- Temperature, pH, Oxygen, Carbon dioxide, Osmotic pressure, Hydrostatic pressure

- Temperature

- Psychrophiles – less than 20 ºC
- Mesophiles – 20 - 45 ºC
- Thermophiles – 45 – 80 ºC
- Extreme thermophiles – more than 85 ºC

- **Psychrotroph- 0-40 (20) ºC**

- **pH (**- log [H+])
  Low pH = acid, High pH = basic or alkaline
- Acidophiles - below pH 5.5 (Acidobacteriota, Mucor)
- Neutrophiles – at pH 6 – 8
- Alkalophiles – above pH 8 (Thiohalospira alkaliphila)

- **Molecular oxygen**

Microbe vary greatly in sensitivity to oxygen.

- Aerobes – microbes which require oxygen.
- Facultative anaerobes – microbes which can grow in presence or absence of oxygen.
- Obligate Anaerobes – which do not utilize oxygen and are killed by oxygen.
- Aerotolerant anaerobe is an organism that tolerates the presence of oxygen but does not require it for growth.
- Microaerophiles – required 3 – 15 % oxygen.

Chemical Requirements for Growth: **Oxygen**

O_2 requirements vary greatly

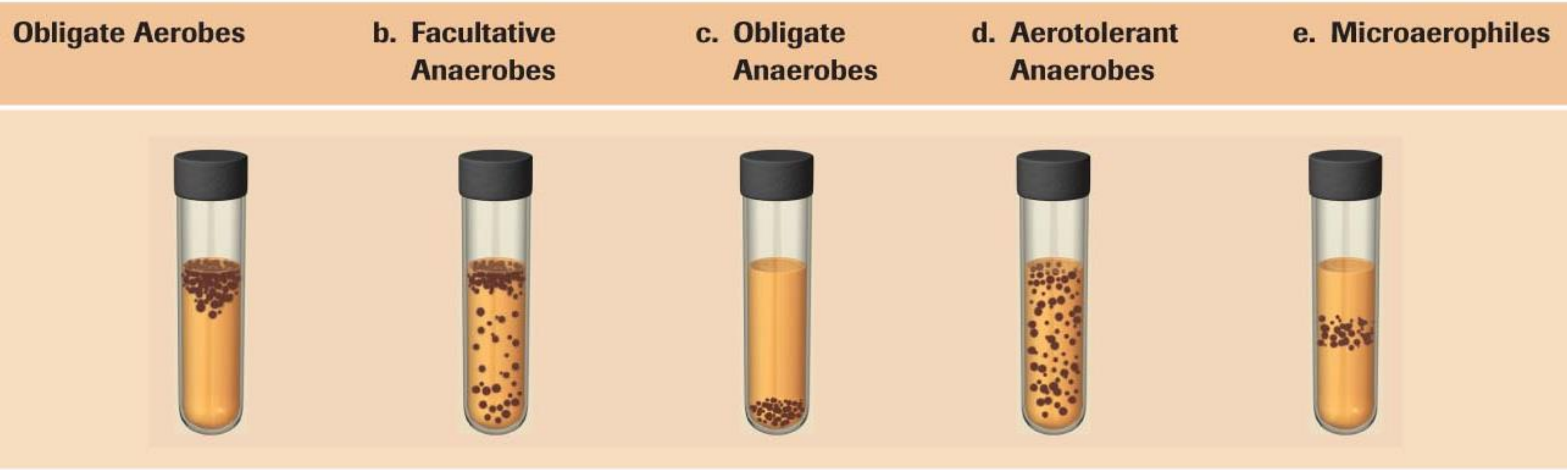


Table 6.1: The Effects of Oxygen on the Growth of Various Types of Bacteria

- Carbon dioxide

- Capnophiles : 3 – 10 % carbon dioxide
- Many microaerophiles are also capnophiles

Osmotic Pressure

(d) Hypotonic (hypoosmotic) solution—water moves into the cell and may cause the cell to burst if the wall is weak or damaged (osmotic lysis)

(e) Hypertonic (hyperosmotic) solution—water moves out of the cell, causing its cytoplasm to shrink (plasmolysis)

Osmotic Pressure

High osmotic pressure (hypertonic) removes water causing plasmolysis — inhibits growth i.e. salt as preservative

Low osmotic pressures (hypotonic) cause water to enter and can cause lysis

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Quantitative methods for measuring growth of bacteria:

The growth of bacteria can be determined by numerous techniques based on one or more of the following types of measurement:

1- **Cell count**

a- microscopy or by using electronic particle counter.

b- colony count or number (plate count method, MPN)

2- **Cell mass**

a- weighting (dry weight)

b- Measurement of cell nitrogen

c- Indirectly by turbidity with culture , directly without culture

3- **Cell activity-** indirectly by relating the degree of biochemical activity to the size of population Such as measurement of utilizing O2

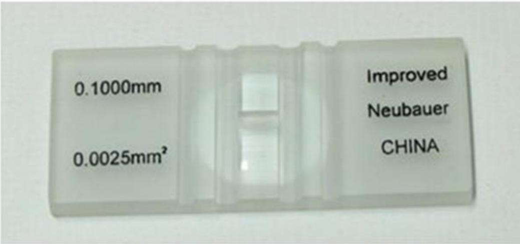
Direct methods :

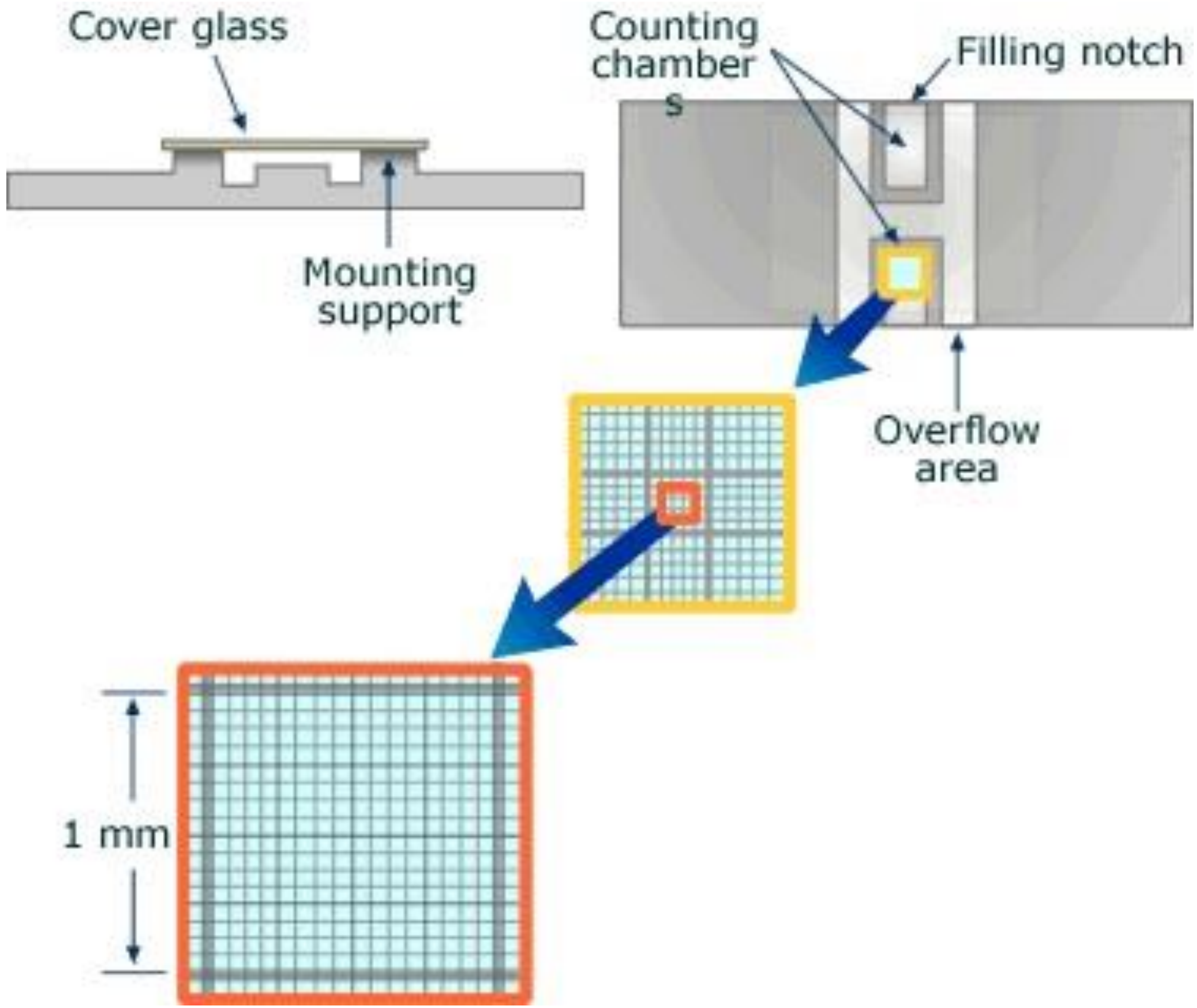
With direct methods we count individual cells or colonies that are assumed to have apart or arise in through the division of a single cell.

1- Counting Chamber (Hemocytometer) :

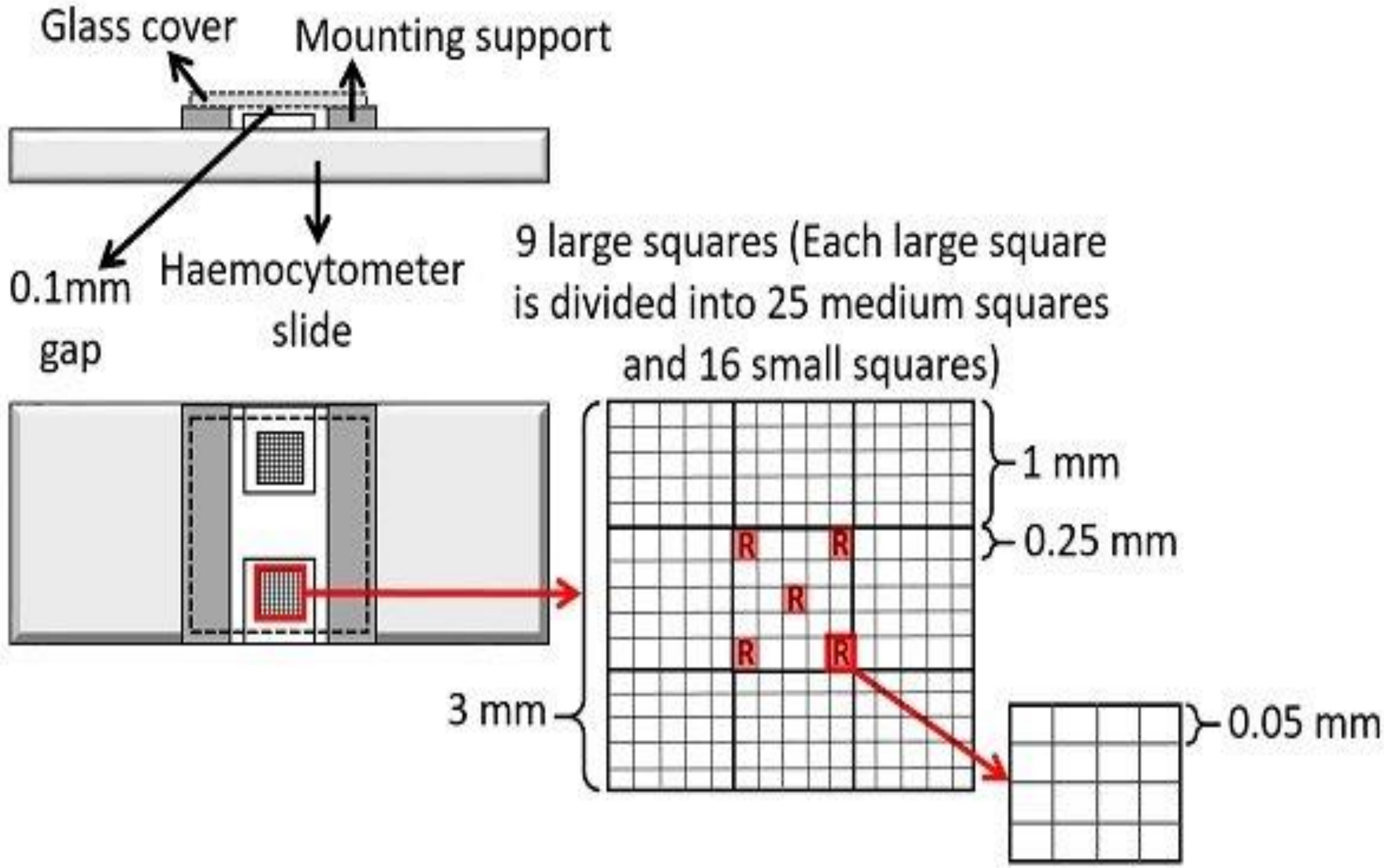
The hemocytometer is a specialized microscope slide used to count cells.

The center portion of the slide has etched grids (H) with precisely spaced lines.

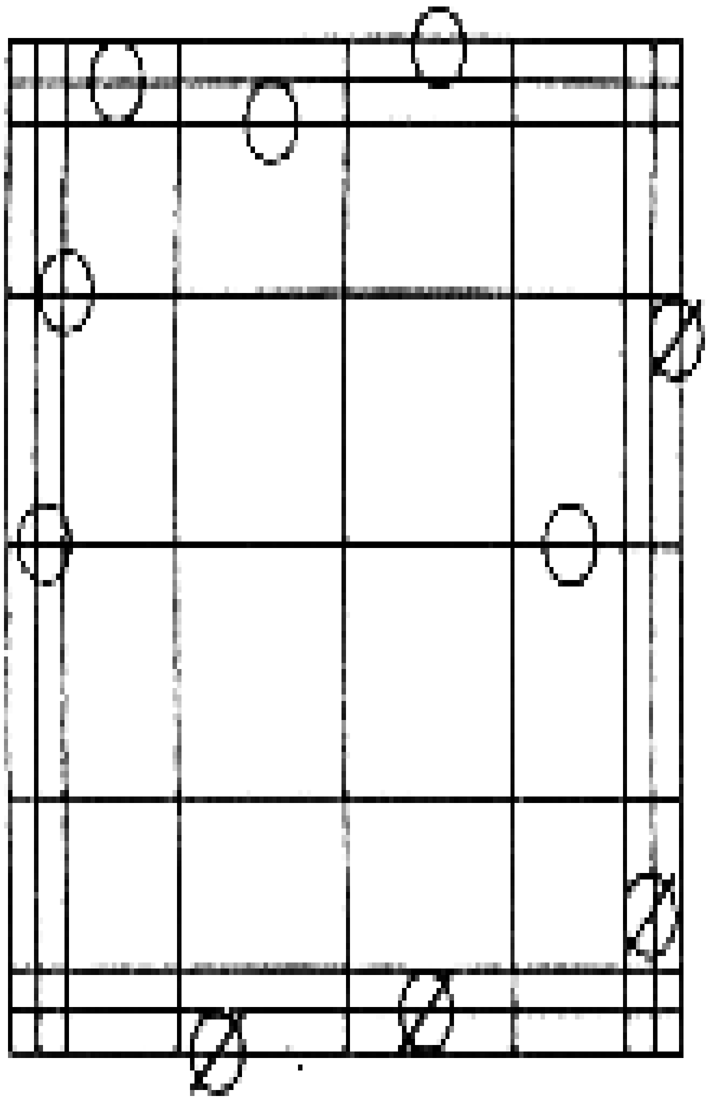


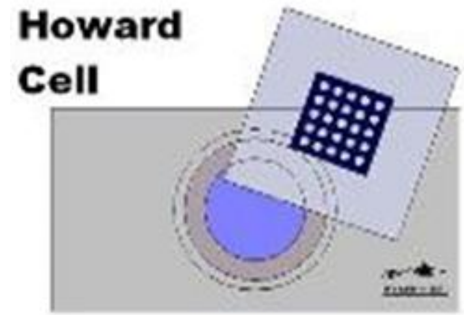

Cover glass
Mounting support
Counting chambers
Filling notch
Overflow area
1 mm

Neubauer's Chamber or Haemocytometer



CORNER SQUARE (ENLARGEMENT)





Count cells on top and left touching middle line (○). Do not count cells touching middle line at bottom and right (Ø).

Steps of Counting of cell using hemocytometer

1- percentage of viable cell= Total viable cell/ total cell *100

2-Average of cell per square = total viable/ average

3- Dilution factor =Final volume / volume of cell

4- Concentration (viable cells/ ml)=Average of cell* dilution factor*

10000 (10^4)

2- Coulter Counter :

electronic counting (this machine detects the difference in current as individual microorganisms pass through a small orifice).

It is Very fast , easy to use but;

Very EXPENSIVE.

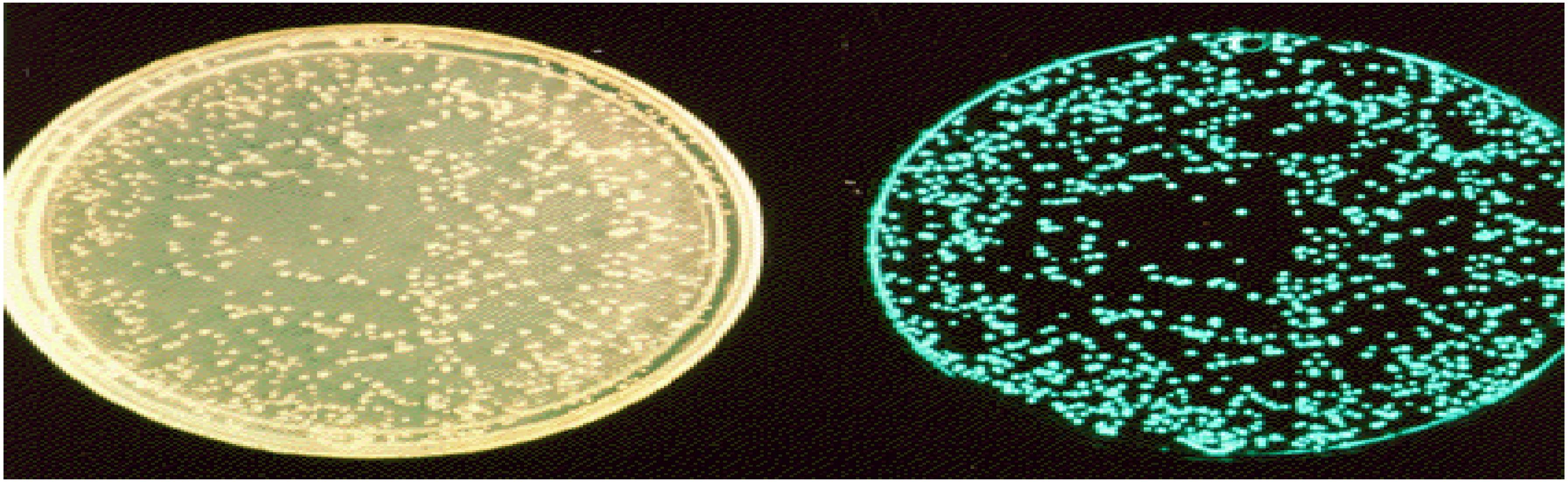
3- Viable count assays (Colony Counting) :

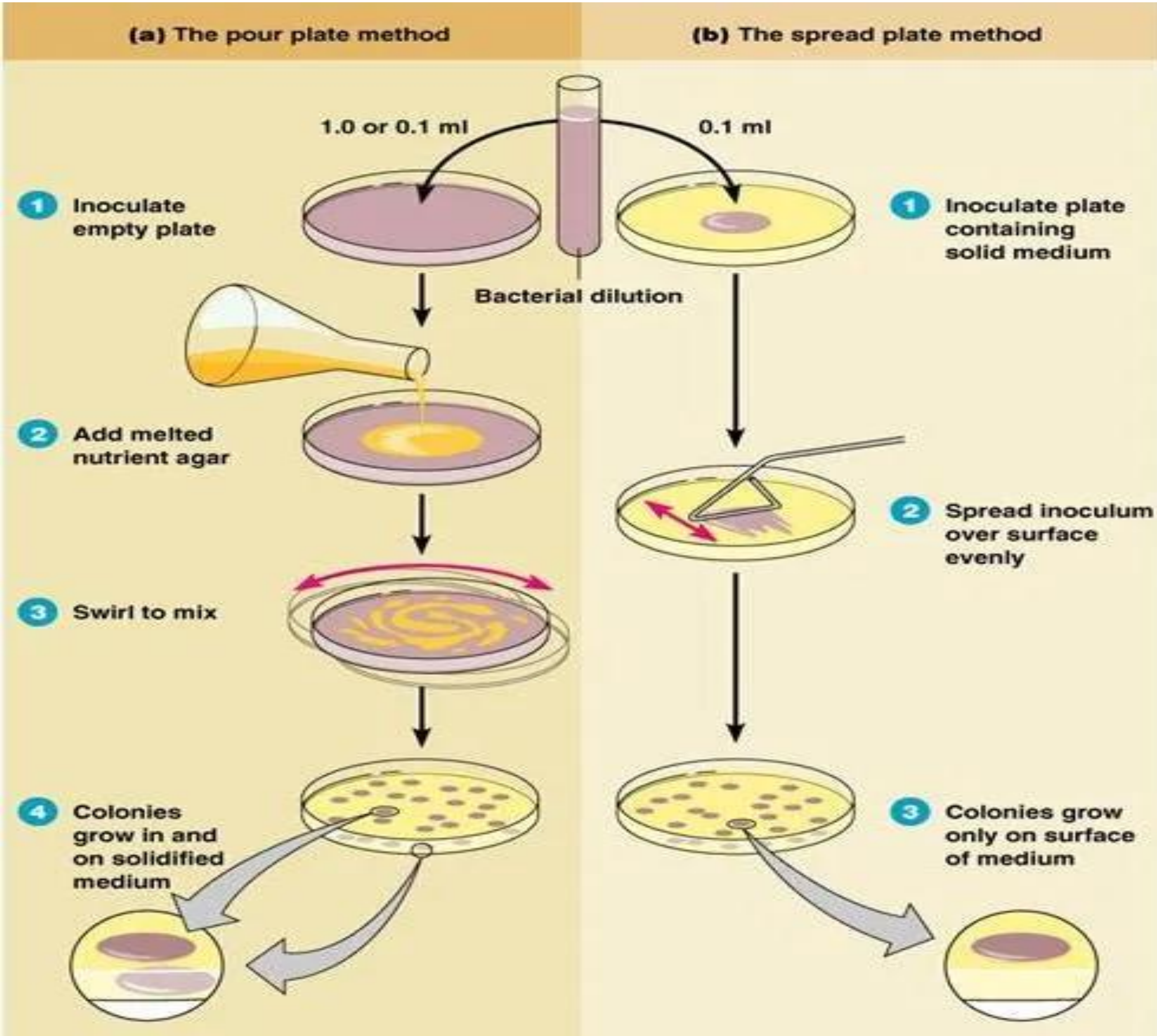
Colony counting after plating dilutions of the sample onto growth medium.

Standard plate counts using spread and pour plate techniques (cfu for “colony forming unit”) .

two viable count assays:

1 - Spread plates 2- Pour plates

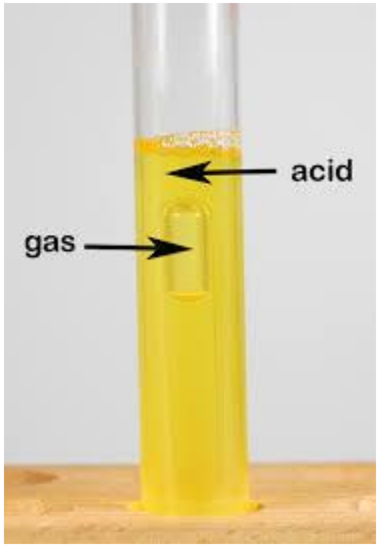



(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
Bacterial dilution
1 Inoculate plate containing solid medium
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium

Indirect Method :

Indirect methods often rely on the results of metabolic tests or other growth characteristics. And it's to:

- Measurement of metabolic activity.
  - Gas or Acid Production.



- Turbidity using a spectrophotometer.

spectrophotometry, using a spectrophotometer .

These Indirect counts depend on:

- The effects of the organisms to estimate their numbers.
- As organisms grow they make the nutrient broth turbid.
- This turbidity can be measured with a colorimeter

Microbial Metabolism

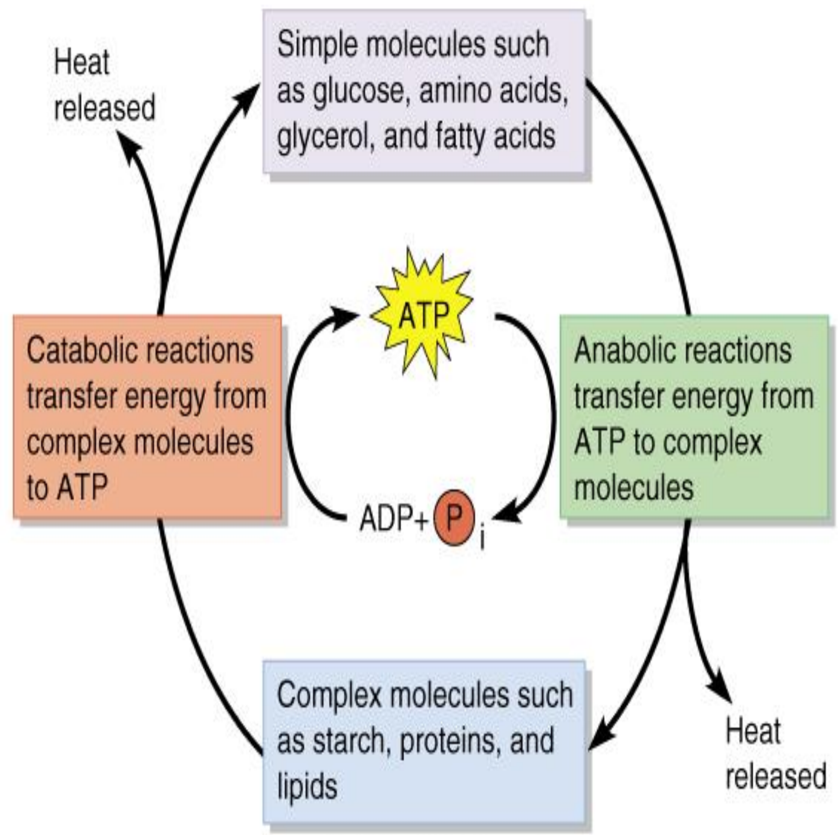
Metabolism: Is the sum of all chemical reactions in the body.

metabolism is divided into two types of classes: **catabolism** and **anabolism**.

- Catabolism is the chemical reactions that break down large compounds and release energy.
- Anabolism is the chemical reactions that require energy to build large compound

- Catabolic reactions furnish the energy needed to drive anabolic reactions.

Energy harvested from catabolic reactions are stored in ATP molecules. ATP molecules are used to drive many anabolic reactions.



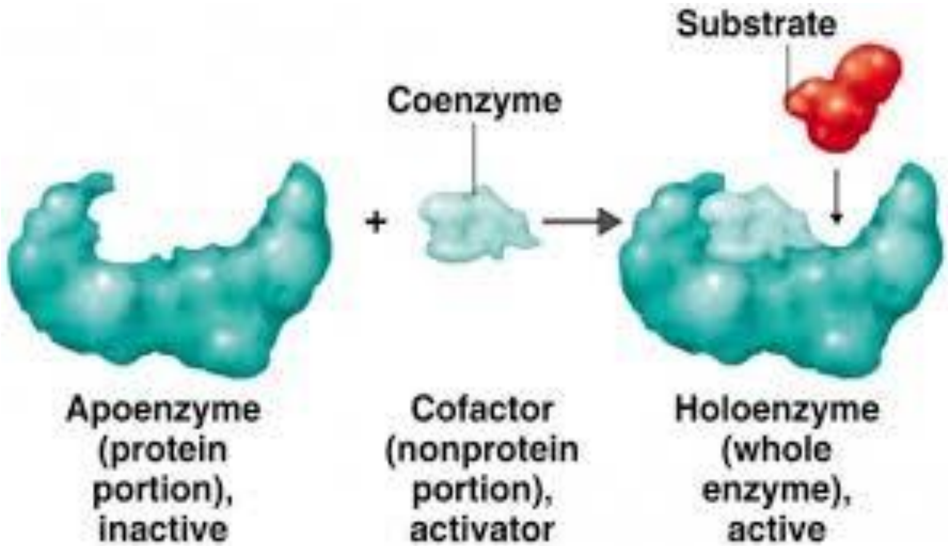
Enzymes

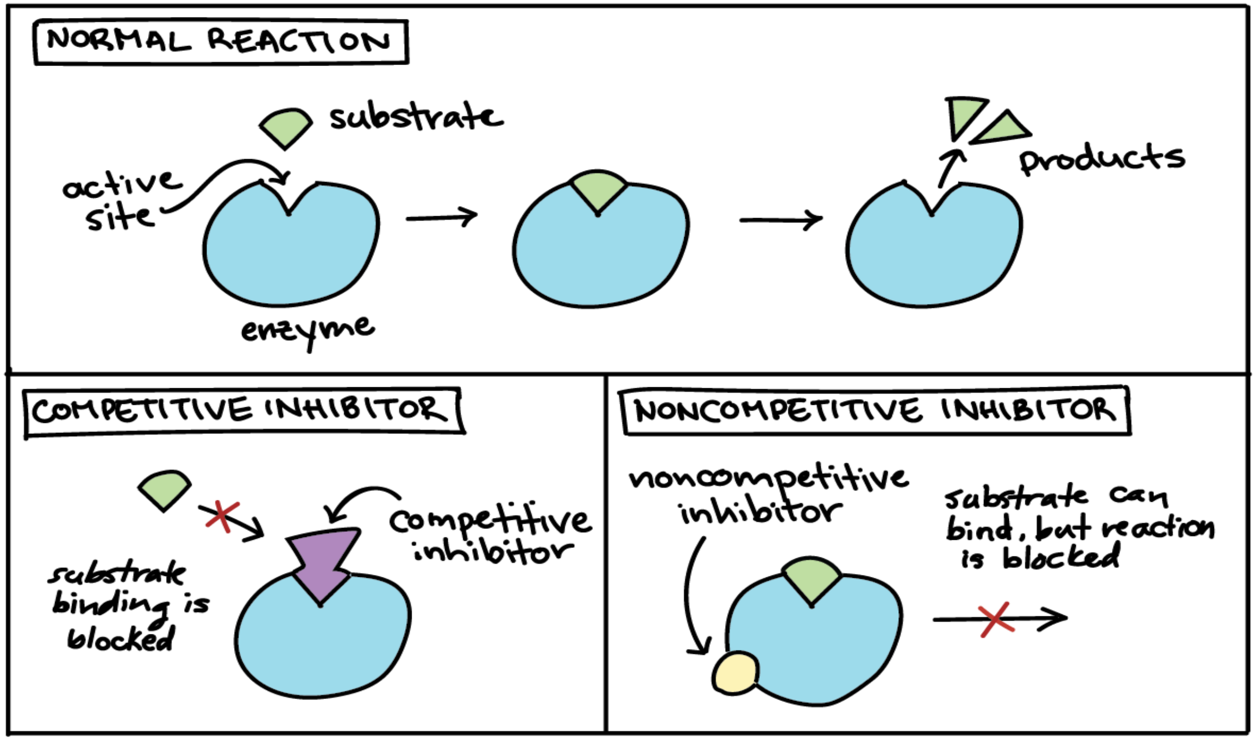
Enzymes are proteins, produced by living cells, that catalyze chemical reactions by lowering the activation energy.

Enzyme Components

1. Most enzymes are holoenzymes, consisting of a protein portion (apoenzyme) and a nonprotein portion (cofactor).
2. The cofactor can be a metal ion (iron, copper, magnesium, manganese, zinc, calcium, or cobalt) or a complex organic molecule known as a coenzyme.
3. Important coenzymes include: nicotinamide adenine dinucleotide (NAD^+), : nicotinamide adenine dinucleotide phosphate ($NADP^+$), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and coenzyme A (CoA).

- **Factors Influencing Enzymatic Activity**
- 1. At high temperatures, enzymes are denatured and lose their catalytic properties; at low temperatures, the reaction rate decreases.
- 2. The pH at which enzymatic activity is maximal is known as the optimum pH.
- 3. Within limits, enzymatic activity increases as substrate concentration increases.
- 4. Competitive inhibitors compete with normal substrate for the active site of the enzyme. (see Example of Competitive Inhibitor)
- 5. Noncompetitive inhibitors act on other parts of the apoenzyme or on the cofactor and decrease the enzyme's ability to combine with substrate.





Penicillin, for **example**, is a **competitive inhibitor** that blocks the active site of an **enzyme** that many bacteria use to construct their cell

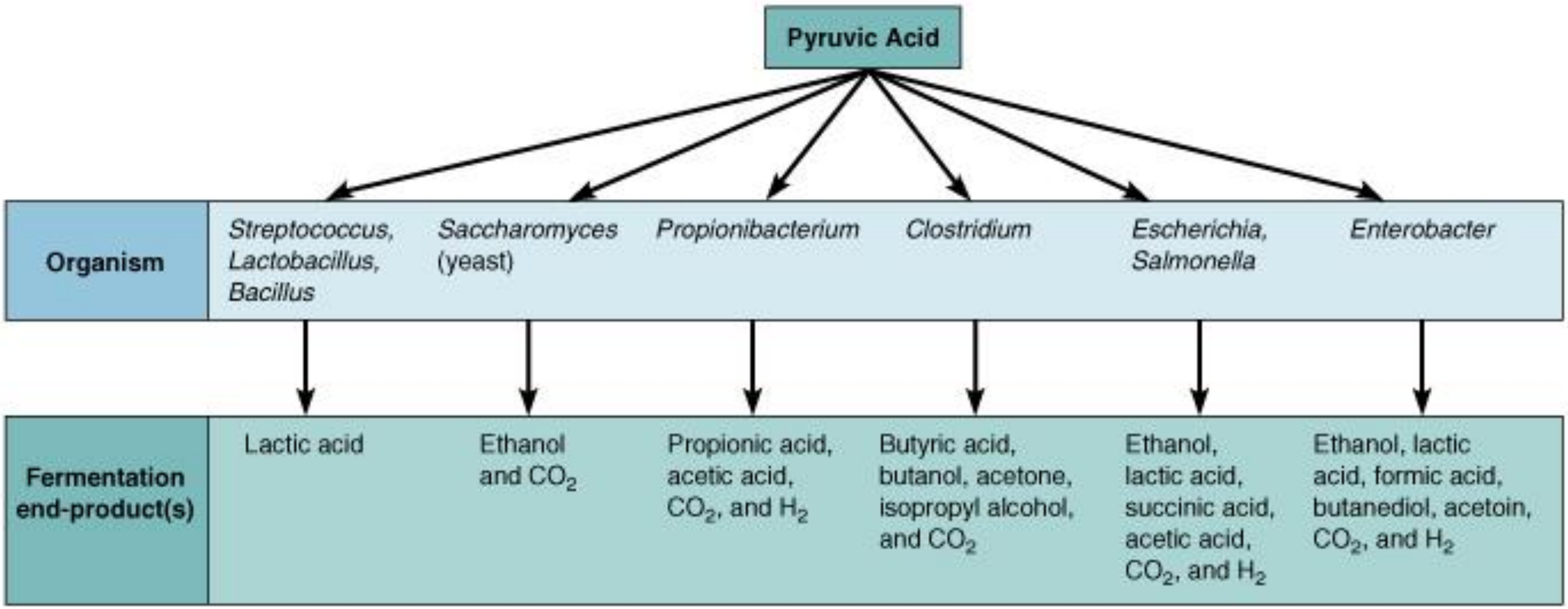
For **example**, the amino acid alanine noncompetitively inhibits the enzyme pyruvate kinase.

Prokaryotic vs Eukaryotic

- Prokaryotic: Remember has no nucleus or no membranes around their organelles. So where does Aerobic Respiration occur here?

Prokaryotic Cells	Eukaryotic Cells
Glycolysis: Cytoplasm	Glycolysis: Cytoplasm
Krebs Cycle: Cytoplasm	Krebs Cycle: Mitochondria
ETC: Cell Membrane	ETC: Mitochondrial Membrane
Fermentation: cytoplasm	Ferm: Cytoplasm

Prokaryotic cells can yield a maximum of 38 ATP molecules while eukaryotic cells can yield a maximum of 36. In eukaryotic cells, the NADH molecules produced in glycolysis pass through the mitochondrial membrane, which "costs" two ATP molecules information



(b)