ENVE 302
Environmental Engineering Unit Processes

CHAPTER: 2

Composition and classification of microorganisms

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BACTERIA

- principal microorganism used in biological wastewater treatment
- single cell, prokaryotic organism
- their usual mode of reproduction binary fission
- are composed of

  80 % water

  20 % dry material (90% organic, 10 % inorganic)
- approximated emprical formula for the organic fraction (may vary with time and species)

$$C_5H_7O_2N \qquad C_{60}H_{87}O_{23}N_{12}P$$

Typical composition of bacteria cells

Principal inorganic nutrients;

P, N ⟶ macronutrients ,

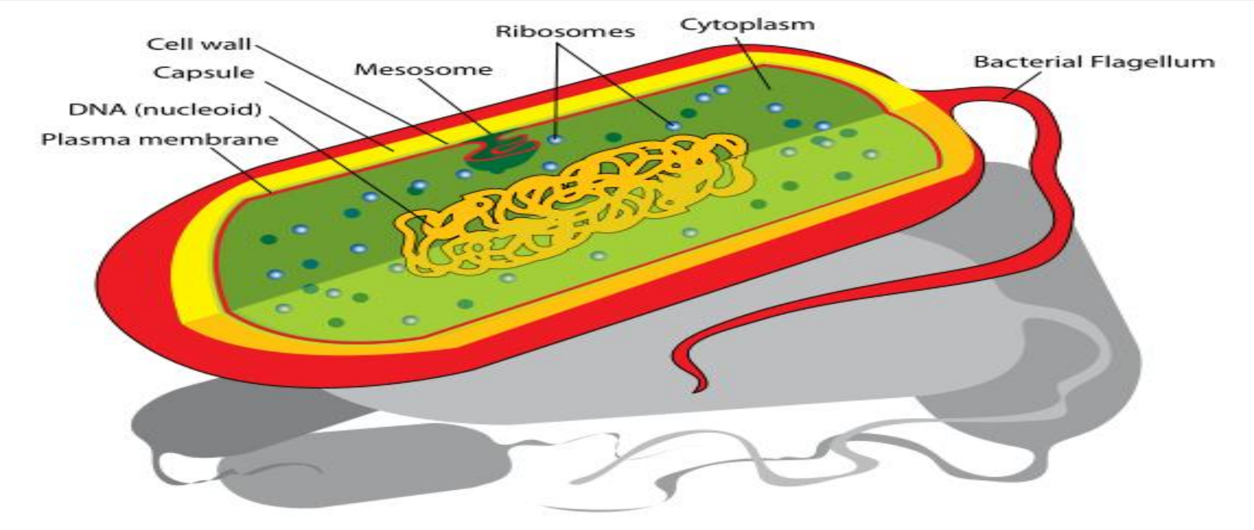
$$C_{60}H_{87}O_{23}N_{12}P$$

12.2 g of nitrogen
2.3 g of phosphorus } are needed per 100 g of cell biomass

S, K, Mg, Ca, Fe, Na, Cl

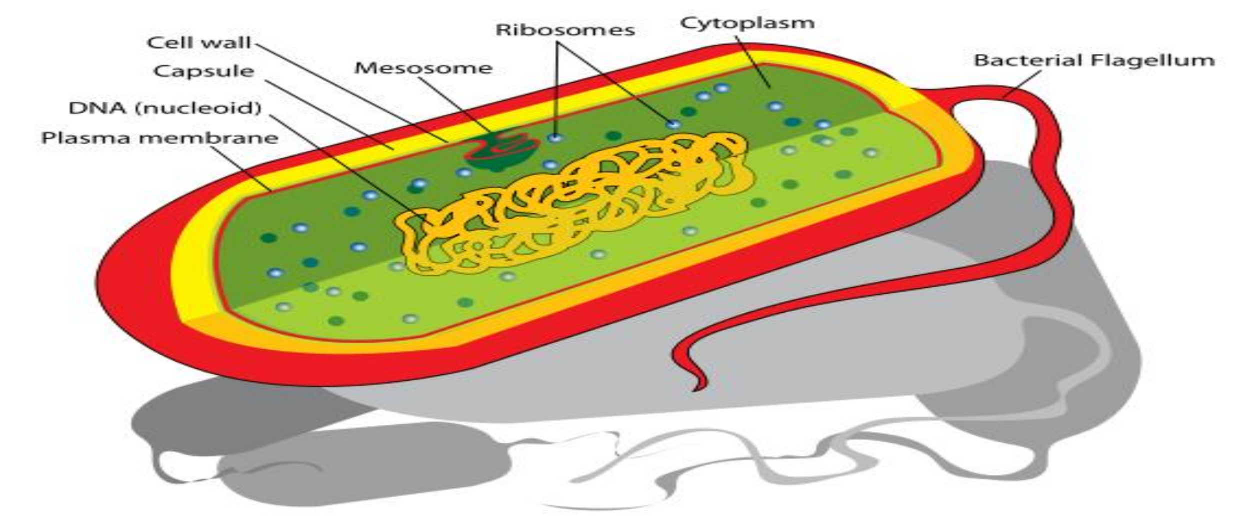
Minor nutrients;
Zn, Mn, Mo, Se, Co, Cu, Ni

Constituent or element	Percent of dry weight
Major cellular material	
Protein	55
Polysaccharide	5
Lipid	9,1
DNA	3,1
RNA	20,5
Other (sugars, amino acids)	6,3
Inorganic ions	1
As cell elements	
Carbon	50
Oxygen	22
Nitrogen	12
Hydrogen	9
Phosphorus	2
Sulfur	1
Potassium	1
Sodium	1
Calcium	0,5
Magnesium	0,5
Chlorine	0,5
Iron	0,2
Other trace elements	0,3



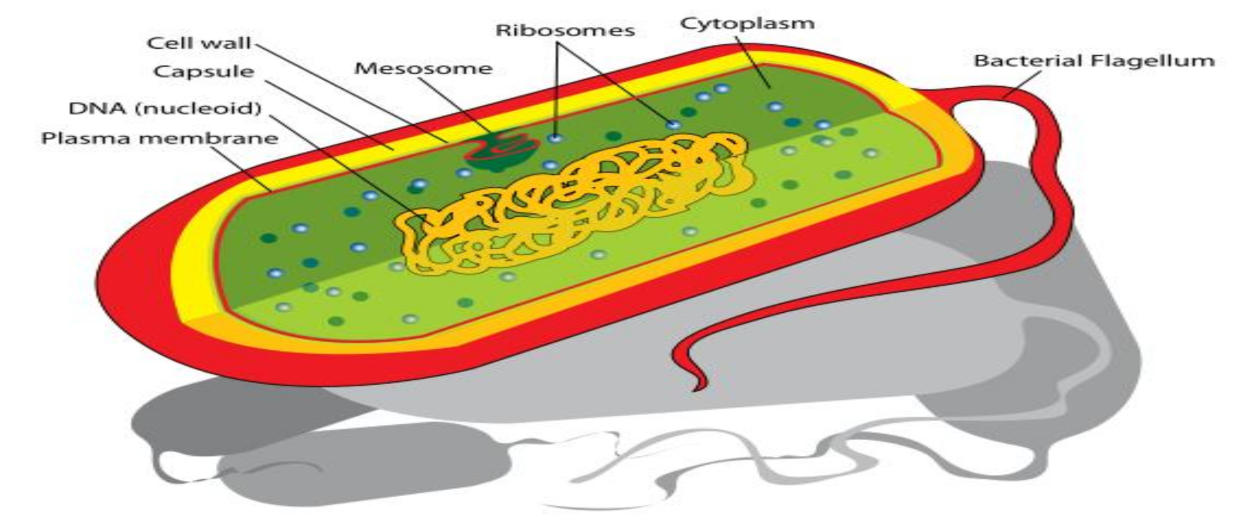
CELL WALL

- confers rigidity to the cell and protects the membrane
- is composed of a repeating building block called peptidoglycan
- some bacteria can produce a sticky polysaccharide layer outside the cell wall, called a capsule or slime layer



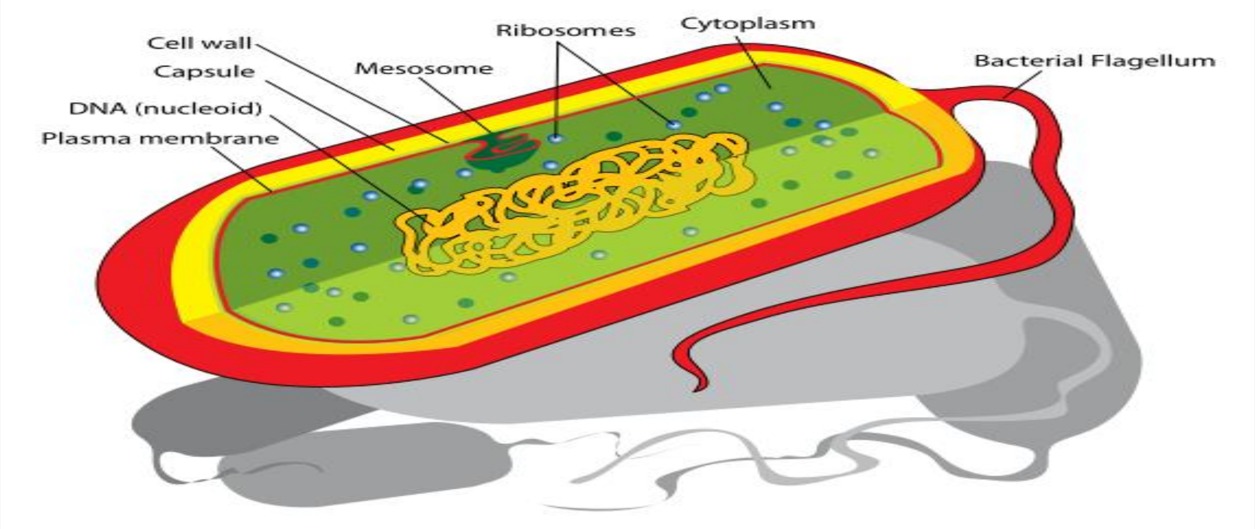
CELL MEMBRANE (CYTOPLASMIC MEMBRANE)

- is a barrier between the cell and its environment
- is semipermeable
- vehicle for restricting what crosses its boundaries
- location of reactions that the cell needs to conduct just outside itself
- is the main barrier to the passage of large molecules
- controls the passage of nutrients into and out of the cell
- location of several important enzymes, including cytochromes, that are involved in electron transport and energy conservation



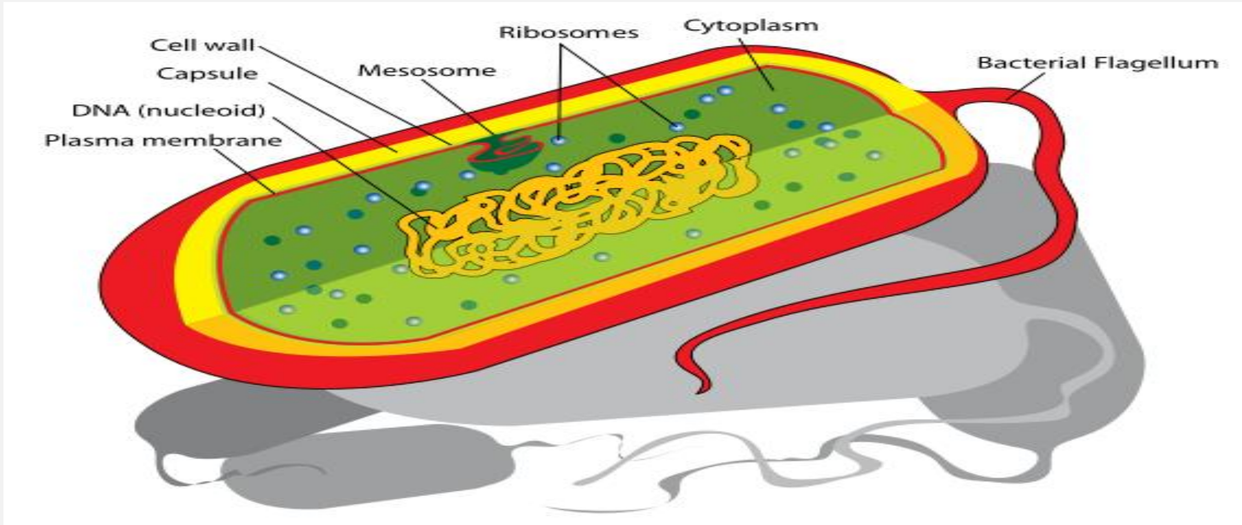
CELL MEMBRANE (CYTOPLASMIC MEMBRANE) (*continue*)

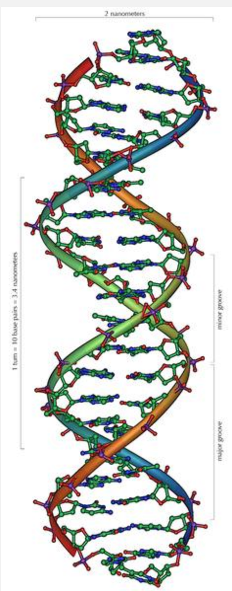
- quite simple in *most bacteria*
- more complex in *autotrophic bacteria*, which synthesize essentially all cellular components from inorganic materials
- even more complex in the *phototrophic bacteria*, which obtain energy from sunlight



CYTOPLASM

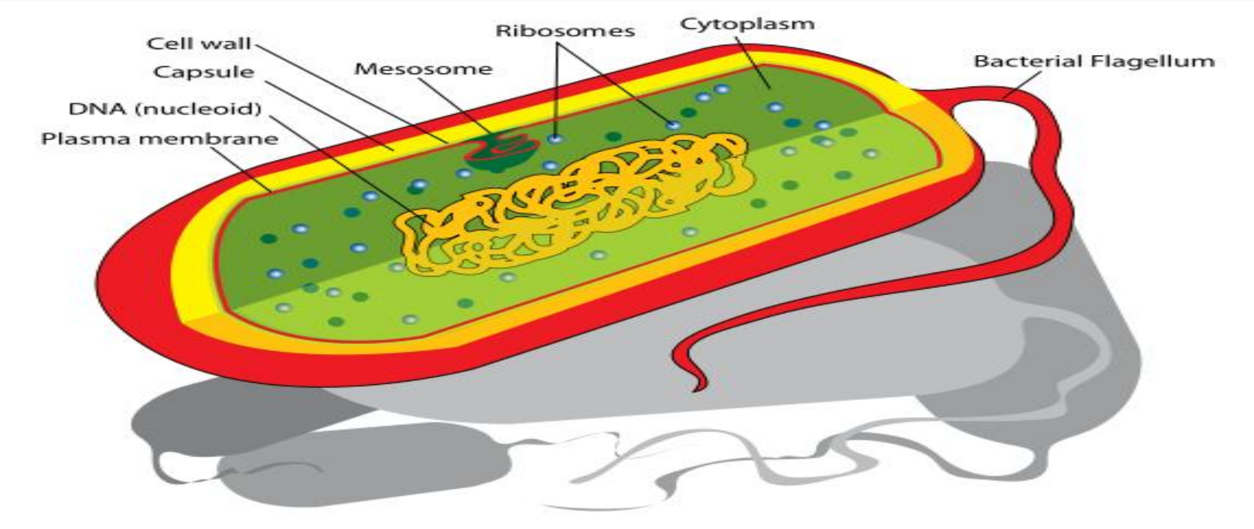
- comprises most of the inside of the cell
- is the material contained within the cell membrane that is used to carry out cell growth and function
- consists of
  - water
  - dissolved nutrients
  - enzymes
  - other proteins
  - the nucleic acids (DNA, RNA)
  - ribosomes (RNA-protein particles containing enzymes for protein synthesis)





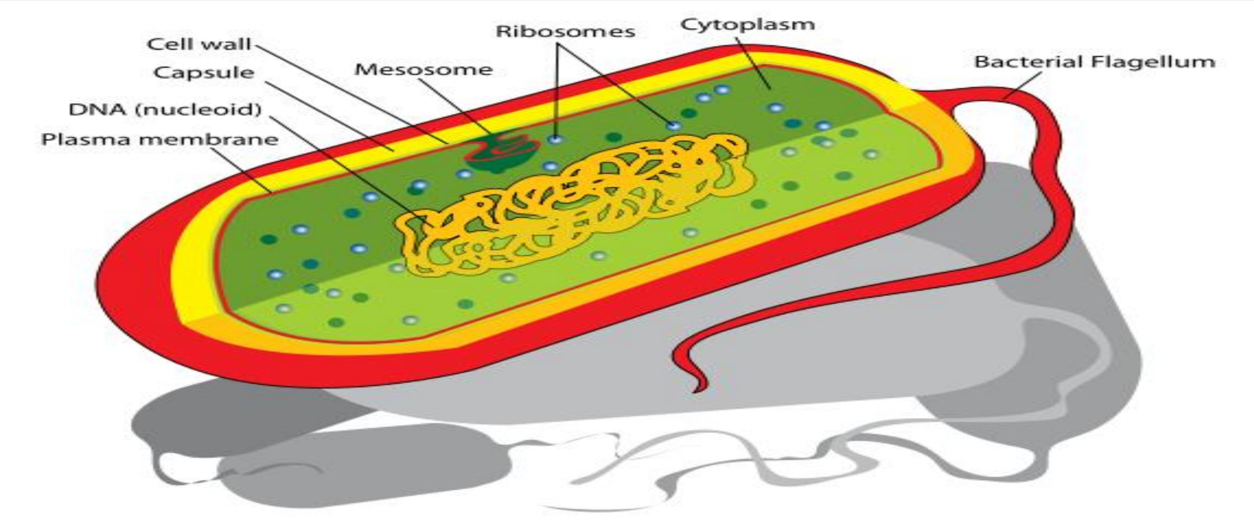
DNA (Deoxyribonucleic acid)

- double-stranded helix shaped molecule
- contains all genetic information required for cell reproduction
- contains in coded form all information required to carry out the normal cell functions
- information is stored in the sequence of nucleotides
- each consisting of deoxyribose connected to one of four nitrogen bases;
  adenine, *guanine*, *cytosine*, *thymine*



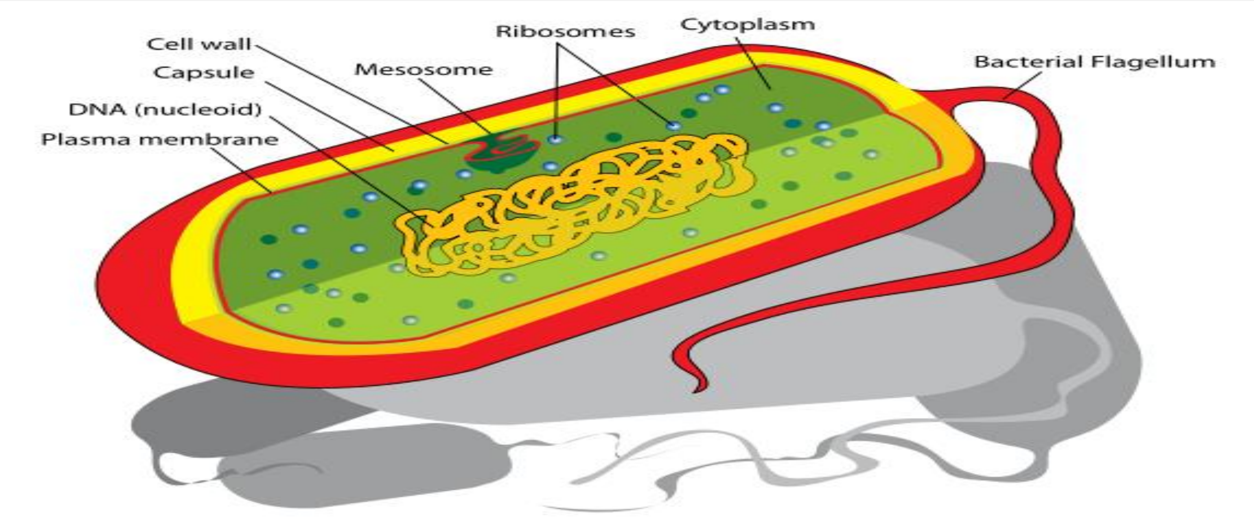
Plasmid DNA

- Small circular DNA molecules
- Provide genetic characteristics for the bacteria



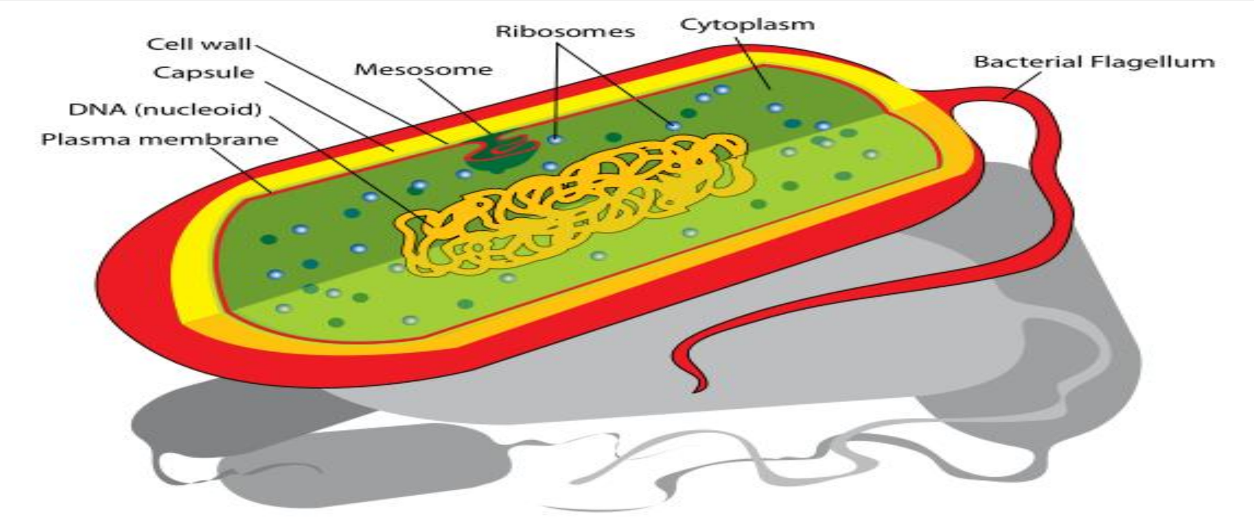
RIBOSOMES

- Particles in the cytoplasm that are composed of RNA and protein
- Sites where the proteins are produced.



FLAGELLA

- Protein hairlike structures that extend from the cytoplasm
- Provide mobility by rotating at high speeds



FIMBRIAE and PILI

- Short protein hairlike structures
- Enable bacteria to stick to surfaces
- Pili enable bacteria to attach to each other

To continue to reproduce and function properly bacteria must have sources of :

- Carbon
- Energy
- inorganic elements (nutrients)

CARBON SOURCE CLASSIFICATION OF BACTERIA

<u>HETEROTROPHS</u>
Organisms that derive cell carbon from organic carbon

<u>AUTOTROPHS</u>
Organisms that derive cell carbon from carbondioxide

The conversion of carbondioxide to cellular carbon compounds → requires a reductive process i.e. requires a net input of energy

Therefore;

Autotrophic organisms → spend more of their energy for synthesis

have lower yields of cell mass and growth rates than do heterotrophs

ENERGY SOURCE CLASSIFICATION OF BACTERIA

<u>PHOTOTROPHS</u>

Energy needed for cell synthesis is suplied by light

<u>PHOTOAUTOTROPH</u>

e.g. algae

<u>PHOTOHETEROTROPH</u>

e.g. sulfur reducing bacteria

<u>CHEMOTROPHS</u>

Energy needed for cell synthesis is supplied by chemical oxidation reactions

<u>CHEMOAUTOTROPH</u>

obtain energy from the oxidation of reduced <u>inorganic compounds</u> (e.g; ammonia, nitrite, nitrate, ferrous iron, sulfide

<u>CHEMOHETEROTROPH</u>

obtain energy from the oxidation of <u>organic compounds</u>

The energy producing reactions by chemotrophs;

oxidation-reduction reactions

electron transfer from electron donor to electron acceptor

electron donor → is oxidized electron acceptor → is reduced

in respiratory metabolism → external electron acceptor is used

in fermantative metabolism → internal electron acceptor is used

less sufficient energy yielding process than respiration

Growth rate and cell yield of strictly fermentative heterotrophic organisms $<$ Growth rate and cell yield of respiratory heterotrophs

Energy- Producing Chemical Reactions

AERORBIC

electron acceptor } O_2

ANOXIC

electron acceptor } NO_2, NO_3

ANAEROBIC

electron acceptor } others e.g, organics, Fe (III) , SO4, CO2

Microorganisms

AEROBIC

obligate aerobes
can only meet their energy needs with oxygen

facultative aerobes
can use oxygen or nitrate/nitrite when oxygen is not available

ANAEROBIC

obligate anaerobes
can exist only in an environment that is devoid of oxygen

facultative anaerobes
have the ability to grow either in the presence or absence of molecular oxygen

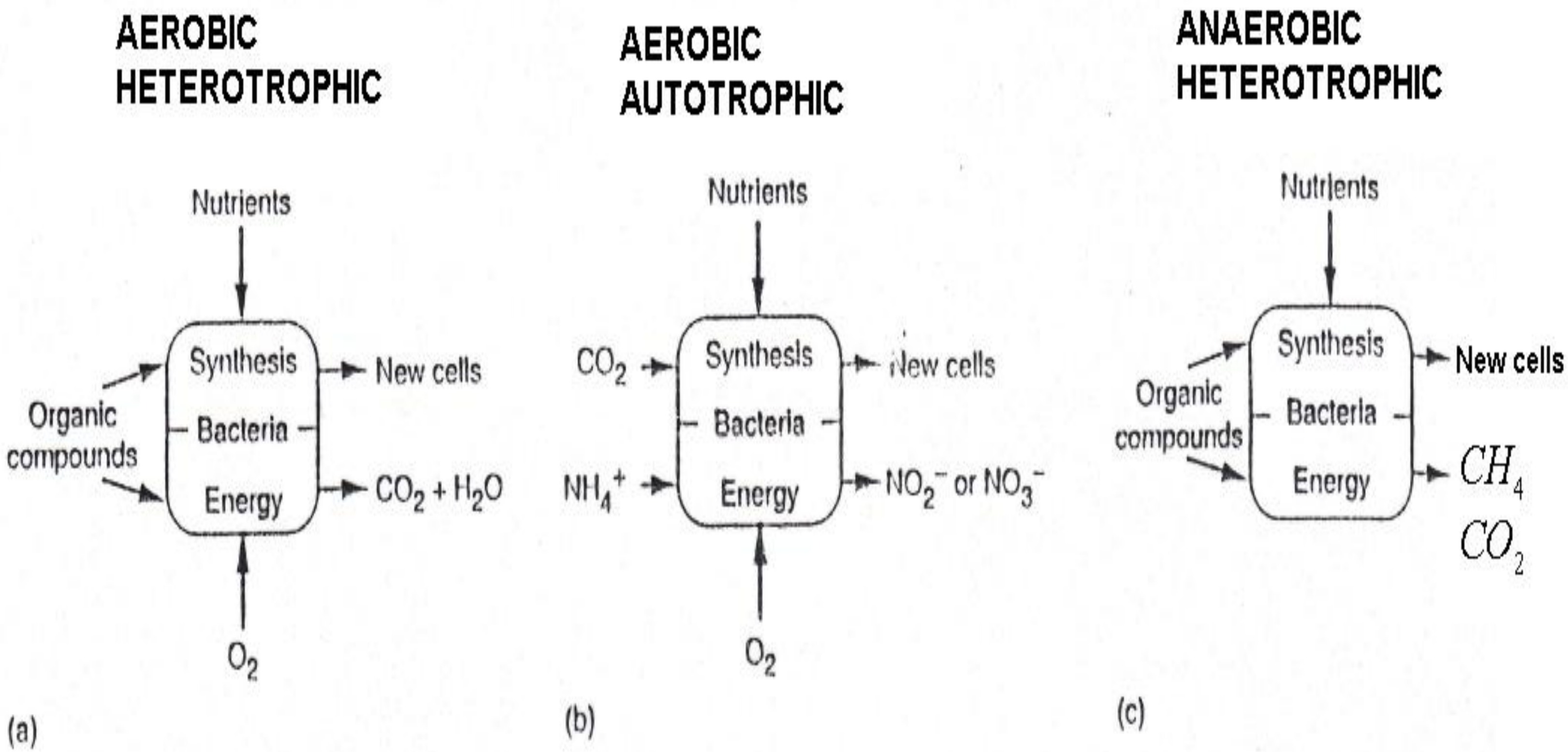


Figure 7-9 Ref: Metcalf & Eddy

Examples of bacteria metabolism: (a) aerobic, heterotrophic, (b) aerobic, autotrophic, (c) anaerobic, heterorophic

Classification of microorganisms by electron, electron acceptor, sources of cell carbon and end products (Ref: Metcalf & Eddy)

	Common reaction name	Carbon Source	Electron Donor	Electron acceptor	End Products
Aeerobic Heterotrophic	Aerobic oxidation	Organic compounds	Organic compounds	O_2	CO_2, H_2O
Aerobic Autotrophic	Nitrification	CO_2	NH_3^- NO_2^-	O_2	NO_2^- NO_3^-
	Iron oxidation	CO_2	Fe(II)	O_2	Ferric Iron, Fe(III)
	Sulfur oxidation	CO_2	H_2S, S, $S_2O_3^{2-}$	O_2	SO_4^{2-}
Facultative Heterotrophic	Denitrification anıxic reaction	Organic compounds	Organic compounds	NO_2^- NO_3^-	N2, CO_2, H_2O
Anaerobic Heterotrophic	Acid fermentation	Organic compounds	Organic compounds	Organic compounds	Volatile fatty acids (VFAs) (acetate,propionate)
	Iron reduction	Organic compounds	Organic compounds	Fe(III)	Fe(II), CO_2, H_2O
	Sulfate reduction	Organic compounds	Organic compounds	SO_4	H_2S, CO_2, H_2O
	Methanogenesis	Organic compounds	Volatile fatty acids (VFAs)	CO_2	Methane

TEMPERATURE CLASSIFICATION OF BACTERIA

psychrophilic
(10 – 30 ^{0}C)
opt: 12-18 ^{0}C

mesophilic
(20 - 50 ^{0}C)
opt: 25 - 40 ^{0}C

thermophilic
(35 - 75 ^{0}C)
opt: 55 -65 ^{0}C

growth rate double with approximately every 10^{0}C increase in temperature until the optimum temperature is reached

Most bacteria can not tolerate pH levels above 9.5 or below 4.

BACTERIAL GROWTH

Reproduction of bacteria → by BINARY FISSION

⇕

Original cell become two new organisms

Time required for each division → GENERATION TIME

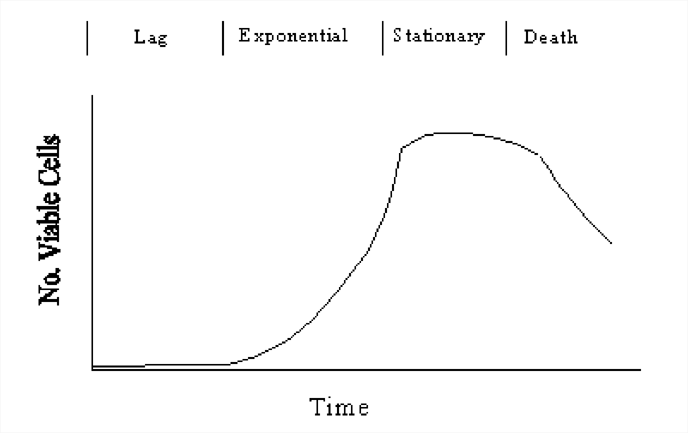
⇕

can vary from days to less than 20 min
depending on species and environmental condition

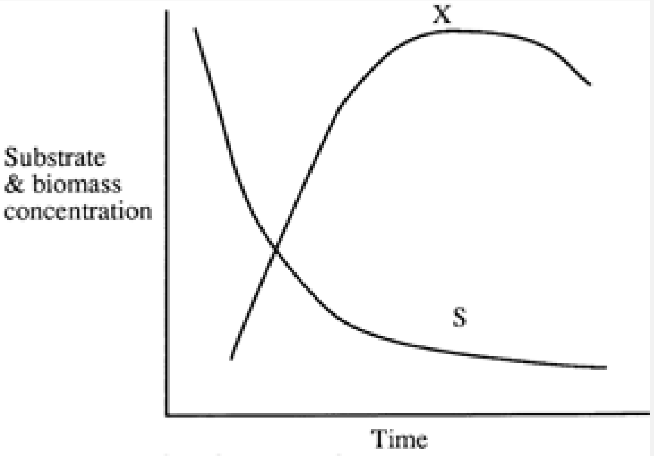
BACTERIAL GROWTH

BACTERIAL GROWTH PATTERNS IN A BATCH REACTOR

Consider the case of a single species of bacteria inoculated in (that is, added to) a medium containig substrate and all nutrients required for growth.



http://www.rocw.raifoundation.org/biotechnology/BTechbiotech/bio-process-engg/lecture-notes/lecture-12.pdf



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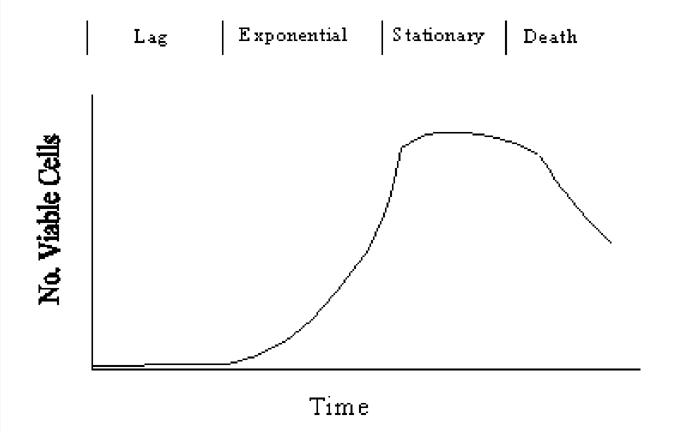
BACTERIAL GROWTH

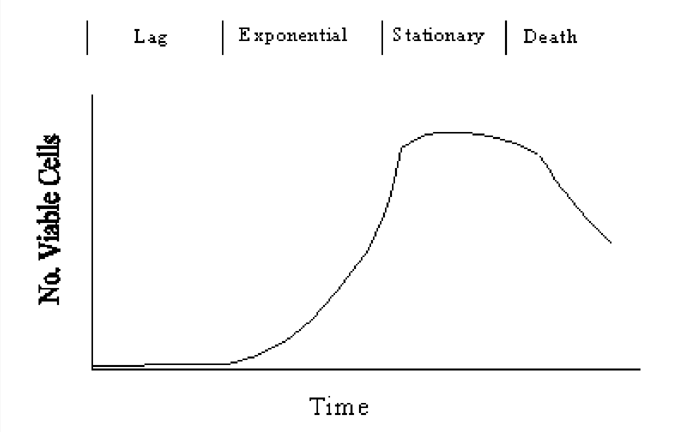


LAG PHASE

- Time required for microorganisms to acclimate to their environment before significant cell division and biomass production occur
- No increase in the number of cells
- Duration of lag phase is greatly dependent upon the age of inoculum culture an the amount of inoculum

If the parent culture is young and biologically active } the lag phase will be extremely short

BACTERIAL GROWTH



EXPONENTIAL GROWTH PHASE (log-growth phase)

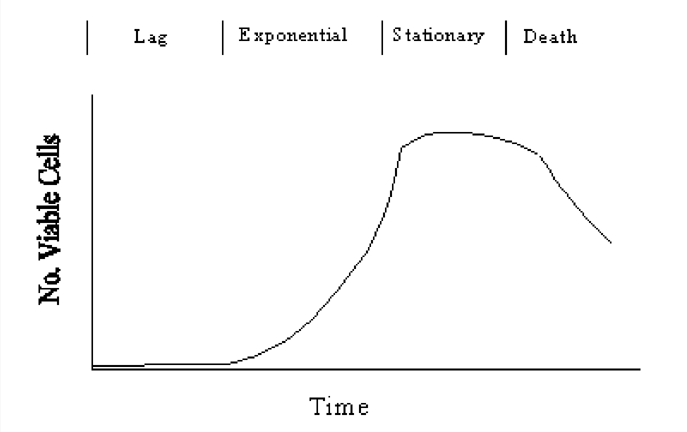
- The rate of fission is the maximum possible as there is no limitation due to substrate or nutrients

- Towards to the end of this phase

  depletion of the substrate or an essential nutrient

  accumulation of toxic end products

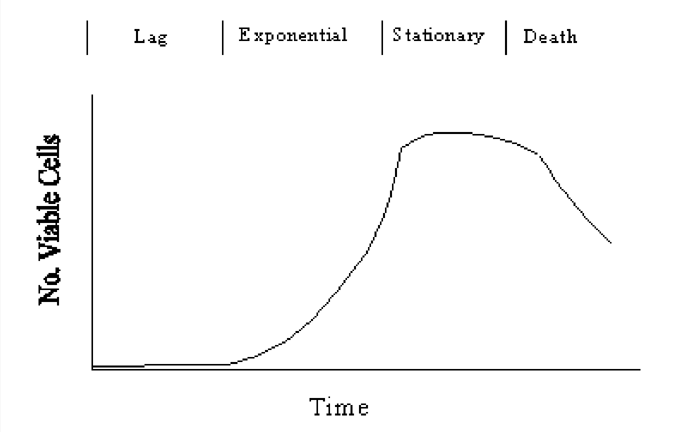
BACTERIAL GROWTH



STATIONARY PHASE

- number of cells dying = number of cells being produced
- bacterial population remains relatively constant with respect to time

BACTERIAL GROWTH



DEATH PHASE

- As the environment becomes more and more adverse to microbial growth (e.g depletion of substrate)

  Cells reproduce more slowly

  Cell die rate exceeds cell growth rate

- Microorganisms start to utilize their own stored food materials and protoplasm in addition to a part of dead cells in the environment as food

- An exponential decline in the biomass concentration

$$\text{BIOMASS YIELD, } Y = \frac{\text{g biomass produced}}{\text{g substrate utilized (i.e consumed)}}$$

for aerobic heterotrohic rxns w/ organic substrates $\longrightarrow$ $Y = \frac{\text{g biomass}}{\text{g organic substrate}}$

for nitrification $\longrightarrow$ $Y = \frac{\text{g biomass}}{\text{g } NH_4\text{-N oxidized}}$

for anaerobic rxns $\longrightarrow$ $Y = \frac{\text{g biomass}}{\text{g VFAs used}}$

Growth Condition	Electron Donor	Electron Acceptor	Synthesis Yield
Aerobic	Organic Compound	Oxygen	0.40 g VSS/g COD
Aerobic	Ammonia	Oxygen	0.12 g VSS/g NH_4-N
Anoxic	Organic Compound	Nitrate	0.3 g VSS/g COD
Anaerobic	Organic Compound	Organic Compound	0.06 g VSS/g COD
Anaerobic	Acetate	CO_2	0.05 g VSS/g COD

Cell yield of anaerobic bacteria degrading organics $<$ Cell yield of nitrifiers $<$ Cell yield of aerobic heterotrophs degrading organic substrate

Anaerobic bacteria degrading organics use internal electron acceptor (fermantative metabolism)

Nitrifiers & aerobic heterotrophs use external electron acceptor (respiratory metabolism)

Cell yield of anaerobic bacteria degrading organics < **Cell yield of nitrifiers** < **Cell yield of aerobic heterotrophs degrading organic substrate**

Anaerobic bacteria (fermantative metabolism) degrading organics use internal electron acceptor

Nitrifiers & aerobic heterotrophs (respiratory metabolism) use external electron acceptor

energy yield of fermantative metabolism < **energy yield of respiratory metabolism** ⇨ **fermantative metabolism lower yields of cell mass and growth rates**

Nitrifiers → autotrophs → use CO_2 as carbon source

conversion of CO_2 to cellular carbon compounds → a reductive process → requires a net input of energy

autotrophs spend great amount of their energy for synthesis → **resulting in lower yields of cell mass and growth rates than aerobic heterotrophs**

Measurement of Biomass

→ VSS

→ Protein Content (50% of biomass dry weight is protein)

→ DNA

→ ATP

VSS

Biomass is mostly organic material→ VSS is chosen as a measure of biomass

VSS is the parameter used most commonly to follow biomass growth in full-scale biological wastewater treatment systems

VSS measurement

→ includes other particulate organic matter in addition to biomass

→ it does not distinguish between living and dead cells

→ its measurement is simple and minimal time is required

The mixture of ww & suspended culture → MIXED LIQUOR

MLVSS → Mixed liquor volatile suspended solids

MLVSS → Mixed liquor suspended solids

Biomass yield
- true yield (synthesis yield) Y
- Observed yield (Y_{obs})

Y_{obs} : based on the actual biomass production and substrate consumption

Synthesis yield (Y) : can be calculated if the stoichiometry of rxn is known

$$\mathbf{Y > Y_{obs}}$$

because a portion of the substrate incorporated into the cell mass will be oxidized with time by the bacteria to obtain energy for cell maintenance

Example: Estimating Biomass Synthesis Yield from Stoichiometry

Assume organic matter can be represented as $C_6H_{12}O_6$ (glucose) and new cells can be represented as $C_5H_7NO_2$. Neglect nutrients other than nitrogen.

$$3\ C_6H_{12}O_6 + 8\ O_2 + 2\ NH_3 \rightarrow 2\ C_5H_7NO_2 + 8CO_2 + 14\ H_2O$$

Oxygen Utilization

For aerobic heterotrophic bacteria;

The quantity of oxygen utilized can be accounted for by considering:

- the oxygen consumed for substrate oxidation to CO_2 and H_2O
- the COD of the biomass
- the COD of any substrate not degraded

Example: Estimating Observed Yield from Field Measurements

The aerobic complete mix biological treatment process without recyle receives ww with a biodegradable soluble COD conc. of 500mg/L. The flowrate is 1000 m^3/d and the reactor effluent biodegradable soluble COD and VSS concentrations are 10 and 200mg/L, respectively. Based on these data

a)What is the Yobs in g VSS/g COD removed ?

b)What is the amount of O_2 used in g O_2/g COD removed and in g/d ?