

Instruction Hours / week: L: 4 T: 0 P: 0 Marks: Internal: 40

External: 60 Total: 100

End Semester Exam: 3 Hours

SCOPE

The students will be able to understand and predict the intermediate metabolism of any microbe used in Industrial production processes. This paper also enables the students about to know about microbial nutrition and growth.

OBJECTIVES

- It Gives brief description on the microbial metabolism and its energetics
- It deals with the various aerobic and anaerobic processes through which the organisms obtain and utilize the energy for their growth.
- Explains photosynthesis and photosynthetic bacteria.

Unit I

Microbial nutrition–nutrient requirements, Nutritional groups of microorganisms. Uptake of nutrients by cell – Passive, Facilitated diffusion, Active transport, Group translocation and Iron uptake.

Unit II

Different phases of growth curve - generation time. Measurement of microbial growth. Batch, Continuous and Synchronous culture, Diauxic growth, Influence of environmental factors on growth (Temperature, pH, solute, water activity, oxygen and pressure).

Unit III

Carbohydrate metabolism – EMP, ED, Pentose phosphate pathway, TCA cycle, Aerobic respiration, oxidative phosphorylation, electron transport chain (Prokaryotic and Eukaryotic), substrate level phosphorylation. Anaerobic respiration. Uncouplers and inhibitors.

Unit IV

Anaerobic respiration with special reference to dissimilatory nitrate reduction (Denitrification; nitrate/nitrite and nitrate/ammonia respiration; fermentative nitrate reduction). Fermentation - Alcohol fermentation and Pasteur effect; Lactate fermentation (homo fermentative and hetero fermentative pathways), concept of linear and branched fermentation pathways

Unit V

Photosynthesis – bacteria and cyanobacteria, photosynthetic pigments – oxygenic (cyanobacterial) and Anoxygenic (Purple, green bacteria) photosynthesis. Nitrogen metabolism-overview of nitrogen cycle.

SUGGESTED READINGS

1. Madigan, M.T., and Martinko, J.M. (2014). Brock Biology of Microorganisms. 14th edition. Prentice Hall International Inc.
2. Moat, A.G., and Foster, J.W. (2002). Microbial Physiology. 4th edition. John Wiley & Sons.
3. Reddy, S.R., and Reddy, M. (2005). Microbial Physiology. Scientific Publishers India.
4. Gottschalk, G. (1986). Bacterial Metabolism. 2nd edition. Springer Verlag.
5. Stanier, R.Y., Ingrahm, J.I., Wheelis, M.L., and Painter, P.R. (1987). General Microbiology. 5th edition, McMillan Press.
6. Willey, J.M., Sherwood, L.M., and Woolverton, C.J. (2013). Prescott's Microbiology. 9th edition. McGraw Hill Higher Education.



KARPAGAM ACADEMY OF HIGHER EDUCATION
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COIMBATORE - 641 021

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

STAFF NAME: Dr. P. AKILANDESWARI

SUBJECT NAME: MICROBIAL PHYSIOLOGY AND METABOLISM

SUB. CODE: 17MBU202

SEMESTER: IV

CLASS: I B. Sc (MB)

S. No	Lecture Duration Period	Topics to be covered	Support material/Page Nos
UNIT - I			
1	1	Microbial physiology and metabolism – Introduction	T1: 99-100
2	1	Microbial nutrition - introduction	
3	1	Nutritional requirements- introduction	
4	1	Nutritional requirements for carbon, hydrogen, oxygen, nitrogen, phosphorous and sulfur	
5	1	Nutritional group of microorganism - phototrophs, chemotrophs, autotrophs, heterotrophs, obligate parasite	T1: 101-103
6	1	Nutritional group of microorganism - heterotrophs, obligate parasite	
7	1	Uptake of nutrients by cell passive	R1: 100
8	1	Uptake of nutrients by facilitated diffusion	R1: 100-104
9	1	Uptake of nutrients by group translocation, active transport	T1: 99-100
10	1	Uptake of nutrition by active transport	
11	1	Uptake of nutrition by Iron uptake	
12	1	Unit Revision	
Total No. of Hours Planned For Unit I=12			
UNIT - II			
S. No	Duration	Topic	Reference
1	1	Different phases of growth - introduction	T1: 119
2	1	Growth curve and growth cycle, Lag phase, Exponential phase, stationary phase, death phase	T1:119-122
3	1	Generation time- transitional periods	
4	1	Quantitative measurement of microbial growth	
5	1	Measurement- direct method, plate count. Pour plate, spread plate, filtration, microscopic	T1:125-128
6	1	Indirect methods: turbidity, metabolic activity, dry	

		weight	
7	1	Bacterial culture - batch culture	
8	1	Bacterial culture - continuous culture, synchronous culture	
9	1	Diauxic growth	
10	1	Influence of environmental factors on growth	
11	1	Temperature, pH, solute, water activity and pressure	
12	1	Unit Revision	
Total No. of Hours Planned For Unit II=12			
UNIT - III			
S. No	Duration	Topic	Reference
1	1	Introduction- carbohydrate metabolism	T1:180
2	1	Embden Meyerhof pathway(EMP, glycolytic) of glucose metabolism	T1:183
3	1	Enter doudoroff pathway(ED) of glucose catabolism	T1:185-186
4	1	Tricarboxylic acid cycle(TCA)	
5	1	Aerobic respiration	
6	1	Oxidative phosphorylation	R1:184-189
7	1	Electron transport chain	
8	1	Substrate level phosphorylation	T1: 190-191
9	1	Anaerobic respiration	
10	1	Uncouplers	
11	1	Inhibitors	
12	1	Unit Revision	
Total No. of Hours Planned For Unit III=12			
UNIT – IV			
S. No	Duration	Topic	Reference
1	1	Anaerobic respiration - Introduction	R1: 190-191
2	1	Dissimilatory nitrate reduction	
3	1	Denitrification	
4	1	Fermentative nitrate reduction	
5	1	Alcohol fermentation	
6	1	Pasteur effect	R1: 189 - 191
7	1	Lactate fermentation	
8	1	Homofermentative pathway	
9	1	Heterofermentative pathway	R1: 190-191
10	1	Concept of linear fermentation pathway	
11	1	Concept of branched fermentation pathway	

12	1	Unit Revision	
Total No. of Hours Planned For Unit IV=12			
UNIT - V			
S. No	Duration	Topic	Reference
1	1	Photosynthesis- Anoxygenic	R1: 468-470
2	1	Oxygenic photosynthesis	R1: 470-473
3	1	Photoreaction at two pigments system	
4	1	Electron carrier in the membrane	
5	1	Phototrophic prokaryotes	
6	1	Purple photosynthetic bacteria, purple sulphuric bacteria	
7	1	Non sulphuric purple bacteria	
8	1	Sulphuric- oxidizing bacteria	
9	1	Sulphur reducing bacteria	
10	1	Nitrogen metabolism	
11	1	Nitrogen cycle	
12	1	Unit Revision	
Total No. of Hours Planned For Unit V=12			

TEXT BOOK

1. Microbiology- Michael J. Pelczar, JR, ECS. Chan and Noel R. Krieg, Tata McGraw-Hill Education Pvt Ltd, 1998.

REFERENCES

1. Microbiology- 5th edition, Lansing M. Prescott, McGraw – Hill Education.
2. Subba Rao, NS. Soil Microbiology, Fourth Edition, Oxford & IBH Publishing Co. Pvt Ltd.

UNIT – 1

MICROBIAL NUTRITION

Microbial nutrition

To obtain energy and construct new cellular components, organisms must have a supply of raw materials or nutrients. Nutrients are substances used in biosynthesis and energy production and therefore are required for microbial growth.

The Common Nutrient Requirements

Analysis of microbial cell composition shows that over 95% of cell dry weight is made up of a few major elements: carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. These are called macro elements or macronutrients because they are required by microorganisms in relatively large amounts. The first six (C, O, H, N, S, and P) are components of carbohydrates, lipids, proteins, and nucleic acids. The remaining four macroelements exist in the cell as cations and play a variety of roles. For example, potassium (K) is required for activity by a number of enzymes, including some of those involved in protein synthesis. Calcium, among other functions, contributes to the heat resistance of bacterial endospores. Magnesium serves as a cofactor for many enzymes, complexes with ATP, and stabilizes ribosomes and cell membranes. Iron is a part of cytochromes and a cofactor for enzymes and electron-carrying proteins. All organisms, including microorganisms, require several micronutrients or trace elements besides macroelements.

The micronutrients—manganese, zinc, cobalt, molybdenum, nickel, and copper are needed by most cells. It is very difficult to demonstrate a micronutrient requirement. In nature, micronutrients are ubiquitous and probably do not usually limit growth. Micronutrients are normally a part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.

Requirements for Carbon, Hydrogen, and Oxygen

The requirements for carbon, hydrogen, and oxygen often are satisfied together.

Carbon is needed for the skeleton or backbone of all organic molecules, and molecules serving as carbon sources normally also contribute both oxygen and hydrogen atoms. They are the source of all three elements. Because these organic nutrients are almost always reduced and have electrons that they can donate to other molecules, they also can serve as energy sources. Indeed, the more reduced organic molecules are the higher their energy content (e.g., lipids have higher energy content than carbohydrates). One important carbon source that does not supply hydrogen or energy is carbon dioxide (CO₂). This is because CO₂ is oxidized and lacks hydrogen. Probably all microorganisms can fix CO₂ that is, reduce it and incorporate it into organic molecules. However, by definition, only autotrophs can use CO₂ as their sole or principal source of carbon. Many microorganisms are autotrophic, and most of these carry out photosynthesis and use light

as their energy source. Some autotrophs oxidize inorganic molecules and derive energy from electron transfers. The reduction of CO₂ is a very energy-expensive process. Thus many microorganisms cannot use CO₂ as their sole carbon source but must rely on the presence of more reduced, complex molecules such as glucose for a supply of carbon. Organisms that use reduced, preformed organic molecules as carbon sources are heterotrophs (these preformed molecules normally come from other organisms). For example, the glycolytic pathway produces carbon skeletons for use in biosynthesis and also releases energy as ATP and NADH. A most remarkable nutritional characteristic of microorganisms is their extraordinary flexibility with respect to carbon sources.

Indigestible molecules sometimes are oxidized and degraded in the presence of a growth promoting nutrient that is metabolized at the same time, a process called cometabolism. The products of this breakdown process can then be used as nutrients by other microorganisms.

Requirements for Nitrogen, Phosphorus, and Sulfur

To grow, a microorganism must be able to incorporate large quantities of nitrogen, phosphorus, and sulfur. Although these elements may be acquired from the same nutrients that supply carbon, microorganisms usually employ inorganic sources as well.

Nitrogen is needed for the synthesis of amino acids, purines, pyrimidines, some carbohydrates and lipids, enzyme cofactors, and other substances. Many microorganisms can use the nitrogen in amino acids, and ammonia often is directly incorporated through the action of such enzymes as glutamate dehydrogenase or glutamine synthetase and glutamate synthase. Most phototrophs and many nonphotosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction. A variety of bacteria (e.g., many cyanobacteria and the symbiotic bacterium *Rhizobium*) can reduce and assimilate atmospheric nitrogen using the nitrogenase system.

Phosphorus is present in nucleic acids, phospholipids, nucleotides like ATP, several cofactors, some proteins, and other cell components. Almost all microorganisms use inorganic phosphate as their phosphorus source and incorporate it directly. Low phosphate levels actually limit microbial growth in many aquatic environments. Phosphate uptake by *E. coli* has been intensively studied. This bacterium can use both organic and inorganic phosphate.

Sulfur is needed for the synthesis of substances like the amino acids cysteine and methionine, some carbohydrates, biotin, and thiamine. Most microorganisms use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction.

Nutritional Types of Microorganisms

In addition to the need for carbon, hydrogen, and oxygen, all organisms require sources of energy and electrons for growth to take place. Microorganisms can be grouped into nutritional classes based on how they satisfy all these requirements.

There are only two sources of energy available to organisms: (1) light energy, and (2) the energy derived from oxidizing organic or inorganic molecules.

Phototrophs use light as their energy source.

Chemotrophs obtain energy from the oxidation of chemical compounds (either organic or inorganic). Microorganisms also have only two sources for electrons.

Lithotrophs (i.e., “rock eaters”) use reduced inorganic substances as their electron source.

Organotrophs extract electrons from organic compounds.

Despite the great metabolic diversity seen in microorganisms, most may be placed in one of four nutritional classes based on their primary sources of carbon, energy, and electrons. The large majority of microorganisms thus far studied are either photolithotrophic autotrophs or chemoorganotrophic heterotrophs.

Photolithotrophic autotrophs (often called photoautotrophs or photolithoautotrophs)

Use light energy and have CO₂ as their carbon source. Eucaryotic algae and cyanobacteria employ water as the electron donor and release oxygen. Purple and green sulfur bacteria cannot oxidize water but extract electrons from inorganic donors like hydrogen, hydrogen sulfide, and elemental sulfur.

Chemolithotrophic autotrophs (chemolithoautotrophs)

These oxidize reduced inorganic compounds such as iron, nitrogen, or sulfur molecules to derive both energy and electrons for biosynthesis. Carbon dioxide is the carbon source. A few chemolithotrophs can derive their carbon from organic sources and thus are heterotrophic. Chemolithotrophs contribute greatly to the chemical transformations of elements (e.g., the conversion of ammonia to nitrate or sulfur to sulfate) that continually occur in the ecosystem. Although a particular species usually belongs in only one of the four nutritional classes, some show great metabolic flexibility and alter their metabolic patterns in response to environmental changes.

Photoorganotrophic heterotrophs

In the absence of oxygen but oxidize organic molecules and function chemotrophically at normal oxygen levels. When oxygen is low, photosynthesis and oxidative metabolism may function simultaneously. They also rely on inorganic energy sources and organic (or sometimes CO₂) carbon sources. These microbes are sometimes called mixotrophic because they combine chemolithoautotrophic and heterotrophic metabolic processes. This sort of flexibility seems complex and confusing, yet it gives its possessor a definite advantage if environmental conditions frequently change.

Chemoorganotrophic heterotrophs (often called chemoheterotrophs, chemoorganoheterotrophs, or even heterotrophs)

Use organic compounds as sources of energy, hydrogen, electrons, and carbon. Frequently the same organic nutrient will satisfy all these requirements. It should be noted that essentially all pathogenic microorganisms are chemoheterotrophs. The other two nutritional classes have fewer microorganisms but often are very important ecologically. Some purple and green bacteria are photosynthetic and use organic matter as their electron donor and carbon source.

Uptake of Nutrients by the Cell

The first step in nutrient use is uptake of the required nutrients by the microbial cell. Uptake mechanisms must be specific that is, the necessary substances, and not others, must be acquired. Since microorganisms often live in nutrient-poor habitats, they must be able to transport nutrients from dilute solutions into the cell against a concentration gradient. Finally, nutrient molecules must pass through a selectively permeable plasma membrane that will not permit the free passage of most substances. In view of the enormous variety of nutrients and the complexity of the task, it is not surprising that microorganisms make use of several different transport mechanisms. The most important of these are passive and facilitated diffusion, active transport, and group translocation. Eucaryotic microorganisms do not appear to employ group translocation but take up nutrients by the process of endocytosis.

Passive diffusion

Except for water and some lipid soluble molecules, few compounds can pass through the cytoplasmic membrane by simple or passive diffusion. In this process, solute molecules cross the membrane as a result of a difference in concentration of the molecules across the membrane. The difference in concentration (higher outside the membrane than inside) governs the rate of inward flow of the solute molecule. With time, this concentration gradient diminishes until equilibrium is reached. In passive diffusion no substance in the membrane interacts specifically with the solute molecules.

Facilitated Diffusion

Another mechanism by which substances cross the semipermeable cell membrane is facilitated diffusion. This process is similar to passive diffusion in that the solute molecules also flow from a higher to a lower concentration. But it is different from passive diffusion because it involves a specific protein carrier molecules (called a porter or permease) located in the cytoplasmic membrane. The carrier molecule combines reversibly with the solute molecule and the carrier solute complex moves between the outer and inner surface of the membrane, releasing one solute molecule on the inner surface and returning to bind a new one on the outer surface. The entry of glycerol into bacterial cells is by facilitated diffusion. Although this mechanism of transport is common in eukaryotic cells (eg, sugars enter them in this way), it is relatively rare in prokaryotic cells.

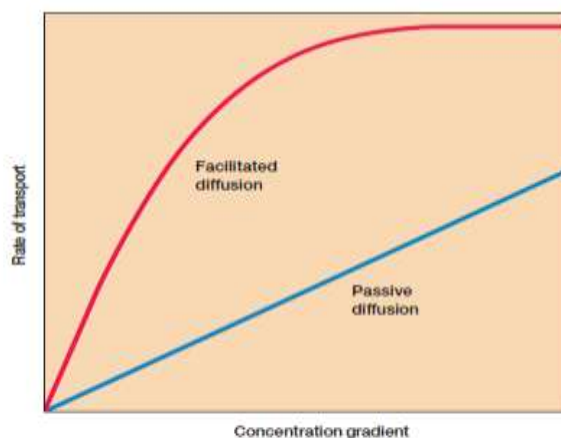


Figure 5.1 Passive and Facilitated Diffusion. The dependence of diffusion rate on the size of the solute's concentration gradient. Note the saturation effect or plateau above a specific gradient value when a facilitated diffusion carrier is operating. This saturation effect is seen whenever a carrier protein is involved in transport.

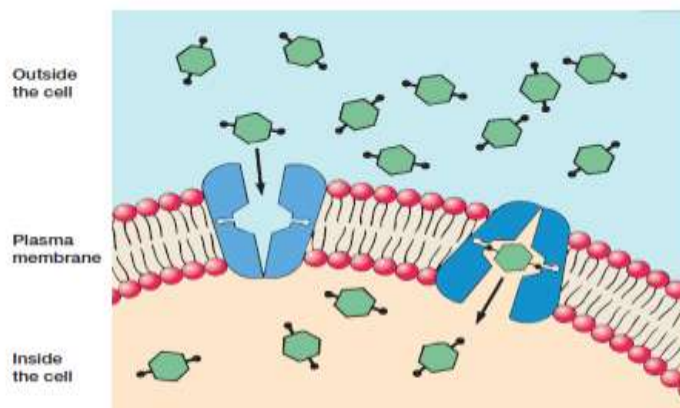


Figure 5.2 A Model of Facilitated Diffusion. The membrane carrier can change conformation after binding an external molecule and subsequently release the molecule on the cell interior. It then returns to the outward oriented position and is ready to bind another solute molecule. Because there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside.

Group Translocation

In active transport, solute molecules move across a membrane without modification. Many procaryotes also take up molecules by group translocation, a process in which a molecule is transported into the cell while being chemically altered (this can be classified as a type of energy-dependent transport because metabolic energy is used). The best-known group translocation system is the phosphoenolpyruvate: sugar phosphotransferase system (PTS). It transports a variety of sugars into procaryotic cells while phosphorylating them using phosphoenolpyruvate (PEP) as the phosphate donor.

Active Transport

Almost all solutes, including sugars, amino acids, peptides, nucleosides and ions are taken by cells through active transport. The three steps of active transport are,

- i) Binding of a solute to a receptor site in a membrane bound carrier protein.
- ii) Translocation of the solute carrier complex across the membrane.
- iii) Coupling of translocation to an energy yielding reaction to lower the affinity of the carrier protein for the solute at the inner membrane surface so that the carrier protein will release solute to the cell interior.

Iron Uptake

Almost all microorganisms require iron for use in cytochromes and many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric iron (Fe_3) and its derivatives, which leaves little free iron available for transport. Many bacteria and fungi have overcome this difficulty by secreting siderophores [iron bearers]. Siderophores are low molecular weight molecules that are able to complex with ferric iron and supply it to the cell. It appears that three siderophore groups complex with iron orbitals to form a six-coordinate, octahedral complex. Microorganisms secrete siderophores when little iron is available in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a siderophore-receptor protein. Then the iron is either released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter. In *E. coli* the siderophore receptor is in the outer membrane of the cell envelope; when the iron reaches the periplasmic space, it moves through the plasma membrane with the aid of the transporter. After the iron has entered the cell, it is reduced to the ferrous form (Fe_2). Iron is so crucial to microorganisms that they may use more than one route of iron uptake to ensure an adequate supply.

Unit – I
Possible Questions

Two Marks

1. Define metabolism.
2. What is microbial nutrition?
3. Mention the common nutrient requirement for microbial growth.
4. What is cofactor?
5. What is cytochrome?
6. Define autotrophs and heterotrophs.
7. What is the function of calcium and magnesium for the microbial growth?
8. Mention the nutritional types of microorganisms.
9. Mention the two sources of energy available for microbial growth.
10. Differentiate between lithotrophs and organotrophs.
11. What is passive and facilitated diffusion?
12. Define active transport.
13. What is siderophore?
14. Define co-metabolism.

Eight Marks

1. Elucidate the classification of microbes based on energy and carbon source.
2. Describe passive diffusion and facilitated diffusion in terms of its distinctive characteristics and mechanisms.
3. Describe the common nutrients required for the growth of microorganisms.
4. Write the need of nitrogen, phosphorus and sulfur for the microbial growth.
5. Explain the nutritional types of microorganisms.
6. Write a brief note on group translocation active transport and iron uptake.

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UNIT I

OPTION A

_____ are organism that make use of carbondioxide as their main source Autotrophy

Bacterial species which grows as phototroph under anaerobic condition and as c *Rhodospirillum rubrum*

Example of bacterial species which can grow either as chemolithotroph or chem *Pseudomonas pseudomonas*

Chemolitho heterotrophs are also called as _____ Mixotroph

Shape of bacterial growth curve is _____ Straight

A solution in which water flows equally in to and out of a cell is termed _____ Isotonic

Members of archaeobacteria that requires high salinity are called as _____ Red extreme halophiles

Shifts in pH in laboratory media can be prevented by incorporating a _____ Acidic solution

A solution in which water flows equally in to and out of a cell is termed _____ Isotonic

The peptidoglycan layer of Gram negative bacteria is located in the ----- sp Triplasmic

_____ is the typical example of bacterium with rod shape. Bacillus megaterium

Organisms that can not utilise oxygen gas are called as _____ obligate Aerobic organisms

Water is important in the nutrition of microorganisms because the food of most Dissolved

Nitrogen is an essential element of the _____ that make up protein Amines

Cyanobacteria resemble green plants in that they evolve _____ as an end product N₂

Lipid content is more in the cell wall of Gram _____ bacteria Negative

Volutin granules are also called as _____ Gas vacuoles

Membrane invaginations in to the bacterial cytoplasm are known as _____ Mesosomes

Microorganisms pathogenic for humans and other warm blooded animals grow at _____ 40°C

In prokaryotic cells the region where DNA is located is referred to as _____ Nucleoid

Semi rigid extension of bacterial cell wall and cell membrane is called _____ Capsule

Gas vesicles are mostly present in _____ Gram positive bacteria

Bacterial ribosomes are composed of _____ Protein and DNA

The nuclear material in a bacterial cell is known as _____ Nucleus

When two molecules are entering the cell simultaneously in the same direction is symport

Phosphorus is essential element of the biosynthesis of _____ as well as A Pyruvic acid

Which of the following mechanisms of transport doesn't involve substrate specific Simple diffusion

Water enters bacterial cell by _____ Facilitated diffusion

Phototransferase system in bacteria is an example of _____ Facilitated diffusion

_____ use light as a source of energy and carbon dioxide as the main source Chemoheterotrophs

95% of cell dry weight is made up of major elements such as C, O, H, N, S and _____ Macro elements

_____ can use CO₂ as their sole source of carbon Chemotrophs

Microbes which grow only in the presence of free oxygen are called Obligate aerobes

Diatoms and many algae are the examples of microorganism requiring vitamin Biotin

_____ is the process in which molecules move from a region of high concentration to low Diffusion

Microorganism capable of growing at zero degree Celsius are called Psychrophiles

_____ are small organic molecules that usually make an all or part of Mineral

Vitamin B6 is otherwise called as _____ riboflavin

The end products of the mixed acid fermentation can be detected by the _____ MR test

Photoautotrophic metabolism using light as the energy source and _____	as Glucose
Permease involved in _____	diffusion
Molecules are modified in _____	group translocation
Ferric ion are _____	soluble
Microorganism use _____ to uptake nutrients.	ferridoxin
Peptone are _____	carbohydrate
Agar is a _____	Protein and DNA
Constituent of cysteine _____	hydrogen
Main constituent of cellular materials _____	hydrogen
Constituent of nucleic acid _____	hydrogen
Cyanobacteria _____	chemoautotrophs
_____ oxidize the inorganic compounds.	lithotrophs
Carbon source for autotrophs _____	organic compounds
_____ use organic form of carbon.	chemoautotrophs
_____ is the inorganic cellular cations.	hydrogen
Which of the following defines a heterotrophs _____	obtain its carbon in a
Molecules that satisfy heterotrophic nutritional requirements include all but which _____	lipid
Primary source of nitrogen for heterotrophs include all except which of the following _____	RNA
Needed in large amount of _____ for cell metabolism	macronutrients
Passive diffusion _____	substance move from
Nitrogen is required for the production of what category of molecules?	Fatty acid

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OPTION B	OPTION C	OPTION D	ANSWER
Heteotroph	Chemotroph	Lithotroph	Autotroph
<i>Rhodospirillum stratu</i>	Proteus	Vibrio	Proteus
<i>Pseudomonas putida</i>	<i>Pseudomonas fluroscer</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomo</i>
Auxotroph	Chemotroph	Lithotroph	Mixotroph
Curved	Sigmoid	Round	Curved
Monomeric	hypertonic	hypotonic	Isotonic
Blue extreme halophi	Green extreme halophil	Yellow extreme halophiles	Red extrer
Normal saline	Physiological saline	Buffer	Buffer
Monomeric	acidic	Neutral	Isotonic
Metaplasmic	Periplasmic	Epiplasmic	Periplasmi
Streptococcus	Corynebacterium	Proteus	Bacillus m
obligate Anaerobic or	Facultatives aerobes	facultative anaerobes	oblicate A
Insoluble	Immersed	Diluted	Dissolved
Aminoacids	Hydroxyl	Carboxyl	Aminoacic
O ₂	CO ₂	H ₂	N ₂
Positive	Both	None	Negative
. Mitochondria	Endoplasmic reticulum	Metachromatic granules	Metachron
Cytosomes	Hydroxysomes	Carboxysomes	Mesosome
37°C	35°C	20°C	37°C
Nuclear region	Nuclear body	Nucleosome	Nuclear re
Stalk	Slime	Prosthecae	Prosthecae
Gram negative bacter	Photosynthetic bacteria	Aquatic bacteria	Aquatic ba
Protein and rRNA	Protein and mRNA	Protein and RNA	Protein an
Nucleoid	Nucleolus	Nucleosome	Nucleoid
antiport	translocation	active transport	symport
Lactic acid	Nucleic acid	Acidic acid	Nucleic ac
Facilitated diffusion	Active transport	Primary active transport	Active trar
Passive diffusion	Active transport	Group translocation	Passive dif
Active transport	Passive diffusion	Group translocation	Passive dif
Chemoautotrophs	Photoautotrophs	Photoheterotrophs	Photoauto
Micro elements	Accessory elements	elements	Macro ele
Heterotrophs	Autotrophs	lithotrophs	Autotroph
Obligate anaerobes	Facultative aerobes	Facultative anaerobes	Obligat
Vitamin B12	Folic acid	Niacin	Vitamin B
Osmosis	Permease	All the above	Diffusion
Acidophiles	Alkalophiles	Mesophiles	Psychroph
Vitamin	Fatty acid	proteins	Vitamin
Cyanocobalimine	Pyridoxine	Ribitol	pyridoxine
VP test	Fermentation test	Nitrate reduction test	MR test

Co ₂	Sucrose	Lactose	Co ₂
Active transport	passive diffusion	facilitated diffusion	Active transport
Active transport	passive diffusion	facilitated diffusion	group transport
insoluble	immiscible	dissolved in water and solvent	insoluble
chlorophyll	siderophores	light	siderophores
lipid hydrolysate	protein hydrolysate	lipid	protein hydrolysate
lipid	Fatty acid	polysaccharide	polysaccharide
sulfur	oxygen	carbon	sulfur
sulfur	oxygen	carbon	carbon
phosphorous	oxygen	carbon	phosphorous
lithotrophs	photoautotrophs	chemotrophs	photoautotrophs
lithotrophs	photoautotrophs	chemotrophs	lithotrophs
inorganic compounds	C	CO ₂	CO ₂
lithotrophs	photoautotrophs	heterotrophs	heterotrophs
oxygen	nitrogen	calcium	calcium
not require essential nutrients	produce all trace elements	produce macronutrients	obtain its nutrients
Protein	water	carbohydrate	water
glucose	DNA	amino acid	glucose
trace elements	micronutrients	nutrients	macronutrients
substance move from outside to inside	substance move from outside to inside	substance move from inside to outside	substance move from outside to inside
phospholipids	Nucleotide	Carbohydrates	Nucleotide

. KEY

y

nas pseudoflava

l

ne halophiles

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egaterium

naerobic organism

ls

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gion

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acteria

d rRNA

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ffusion

ffusion

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carbon in an organic form

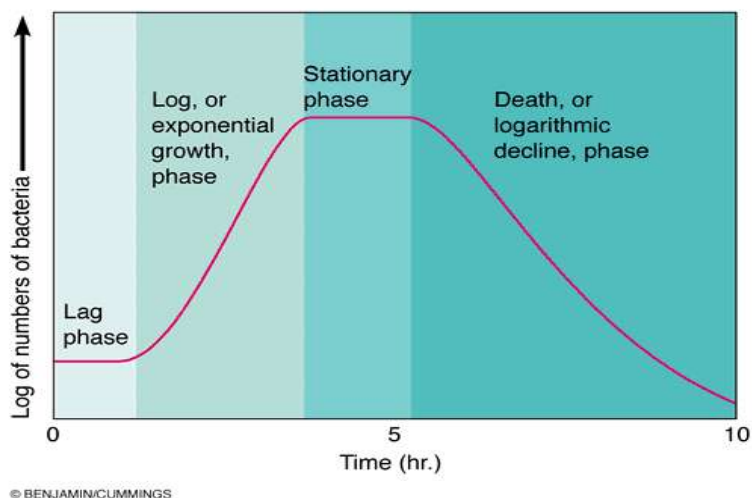
ients
move from higher to lower concentration
s

UNIT – 2**MICROBIAL GROWTH****Microbial Growth**

Growth may be defined as an increase in cellular constituents. It leads to a rise in cell number when microorganisms reproduce by processes like budding or binary fission. In the later, individual cells enlarge and divide to yield two progeny of approximately equal size. Growth also results when cells simply become longer or larger. If the microorganism is coenocytic that is, a multinucleate organism in which nuclear divisions are not accompanied by cell divisions and growth results in an increase in cell size but not cell number. It is usually not convenient to investigate the growth and reproduction of individual microorganisms because of their small size. Therefore, when studying growth, microbiologists normally follow changes in the total population number.

The Growth Curve

Population growth is studied by analyzing the growth curve of a microbial culture. When microorganisms are cultivated in liquid medium, they usually are grown in a batch culture or closed system that is, they are incubated in a closed culture vessel with a single batch of medium. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases.



Microbial Growth Curve in a Closed System

Lag Phase

When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs, and therefore this period is called the lag phase. Although cell division does not take place right away and there is no net increase in mass, the cell is synthesizing new components. A lag phase prior to the start of cell division can be necessary for a variety of reasons. The cells may be old and depleted of ATP, essential cofactors, and ribosomes; these must be synthesized before growth can begin. The medium may be different from the one the microorganism was growing in previously. Here new enzymes would be needed to use different nutrients. Possibly the microorganisms have been injured and require time to recover. Whatever the causes, eventually the cells retool, replicate their DNA, begin to increase in mass, and finally divide. The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase. On the other hand, when a young, vigorously growing exponential phase culture is transferred to fresh medium of the same composition, the lag phase will be short or absent.

Exponential Phase

During the exponential or log phase, microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing. Their rate of growth is constant during the exponential phase; that is, the microorganisms are dividing and doubling in number at regular intervals. Because each individual divides at a slightly different moment, the growth curve rises smoothly rather than in discrete jumps. The population is most uniform in terms of chemical and physiological properties during this phase; therefore exponential phase cultures are usually used in biochemical and physiological studies. Exponential growth is balanced growth. That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, unbalanced growth results. This is growth during which the rates of synthesis of cell components vary relative to one another until a new balanced state is reached. This response is readily observed in a shift-up experiment in which bacteria are transferred from a nutritionally poor medium to a richer one.

Stationary Phase

Eventually population growth ceases and the growth curve becomes horizontal. This stationary phase usually is attained by bacteria at a population level of around 10^9 cells per ml. Other microorganisms normally do not reach such high population densities, protozoan and algal cultures often having maximum concentrations of about 10^6 cells per ml. Of course final population size depends on nutrient availability and other factors, as well as the type of microorganism being cultured. In the stationary phase the total number of viable microorganisms remains constant. This may result from a balance between cell division and cell death, or the population may simply cease to divide though remaining metabolically active. Microbial populations enter the stationary phase for several reasons. One obvious factor is nutrient

limitation; if an essential nutrient is severely depleted, population growth will slow. Aerobic organisms often are limited by O₂ availability. Oxygen is not very soluble and may be depleted so quickly that only the surface of a culture will have an O₂ concentration adequate for growth.

The cells beneath the surface will not be able to grow unless the culture is shaken or aerated in another way. Population growth also may cease due to the accumulation of toxic waste products. This factor seems to limit the growth of many anaerobic cultures (cultures growing in the absence of O₂). For example, streptococci can produce so much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and growth is inhibited.

Death Phase

Detrimental environmental changes like nutrient deprivation and the buildup of toxic wastes lead to the decline in the number of viable cells characteristic of the death phase. The death of a microbial population, like its growth during the exponential phase, is usually logarithmic (that is, a constant proportion of cells die every hour). This pattern in viable cell count holds even when the total cell number remains constant because the cells simply fail to lyse after dying. Often the only way of deciding whether a bacterial cell is viable is by incubating it in fresh medium; if it does not grow and reproduce, it is assumed to be dead. That is, death is defined to be the irreversible loss of the ability to reproduce. Although most of a microbial population usually dies in a logarithmic fashion, the death rate may decrease after the population has been drastically reduced. This is due to the extended survival of particularly resistant cells. For this and other reasons, the death phase curve may be complex.

Measuring Microbial Growth

Direct Methods of Measurement

1. Plate count:

- Most frequently used method of measuring bacterial populations.
- Inoculate plate with a sample and count number of colonies.

Assumptions:

- Each colony originates from a single bacterial cell.
- Original inoculum is homogeneous.
- No cell aggregates are present.

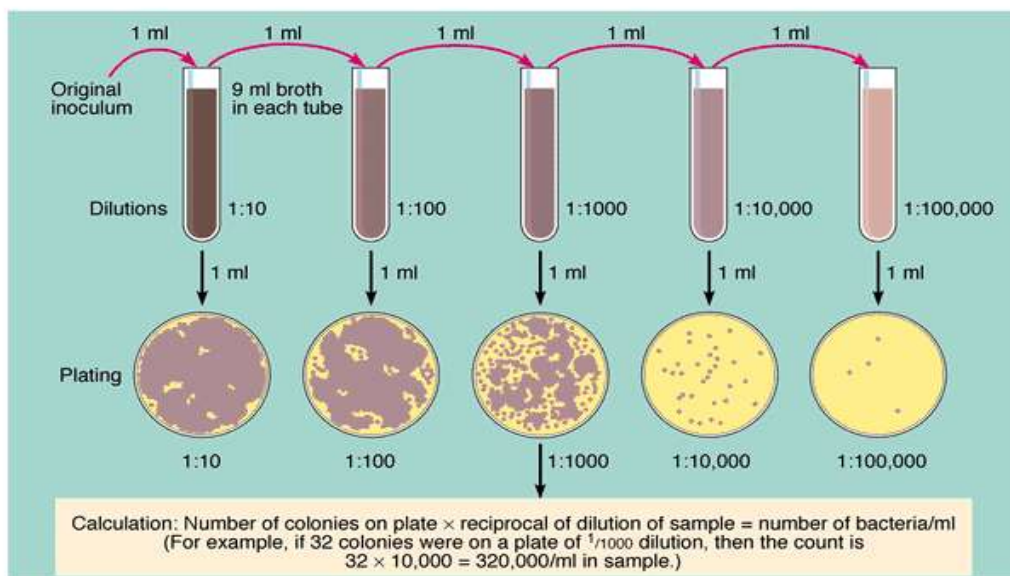
Advantages:

- Measures viable cells

Disadvantages:

- Takes 24 hours or more for visible colonies to appear.
- Only counts between 25 and 250 colonies are accurate.
- Must perform serial dilutions to get appropriate numbers/plate.

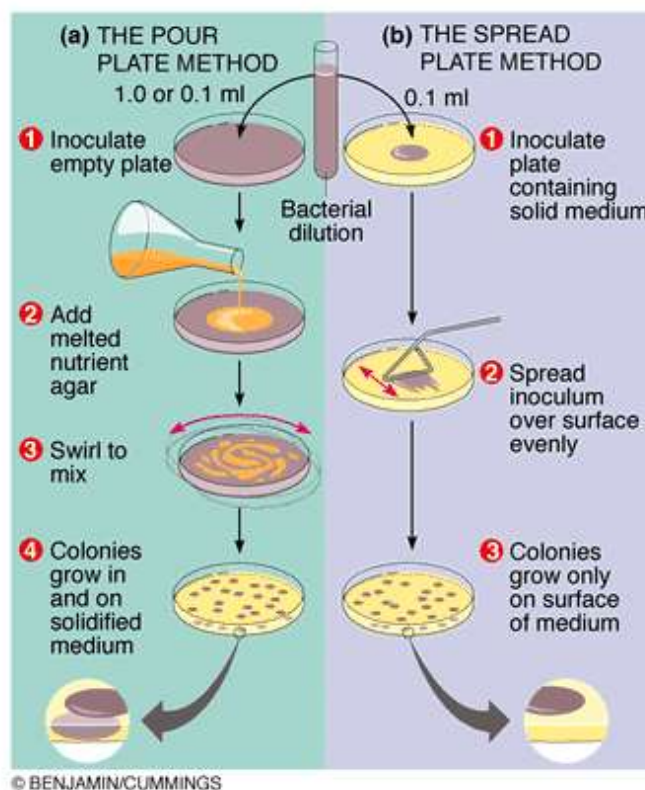
- Serial Dilutions are used with the Plate Count Method to Measure Numbers of Bacteria



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Serial Dilutions are used with the Plate Count Method to Measure Numbers of Bacteria

Pour Plates versus Spread Plates



A. Pour Plate:

- Introduce a 1.0 or 0.1 ml inoculum into an empty Petri dish.
- Add liquid nutrient medium kept at 50°C.
- Gently mix, allow to solidify and incubate.

Disadvantages:

- Not useful for heat sensitive organisms.
- Colonies appear under agar surface.

B. Spread Plate:

- Introduce a 0.1 ml inoculum onto the surface of agar medium containing in the Petri dish.
- Spread with a sterile glass rod.
- **Advantages:** Colonies will be on surface and not exposed to melted agar.

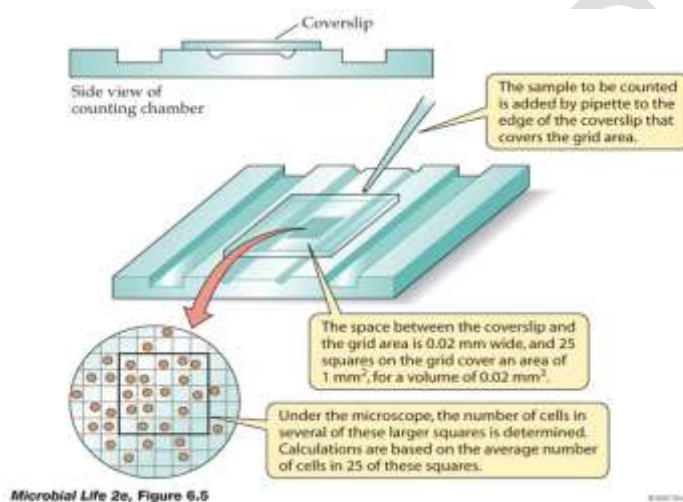
2. Filtration:

- Used to measure small quantities of bacteria.
Example: Fecal bacteria in a lake or in ocean water.
- A large sample (100 ml or more) is filtered to retain bacteria.
- Filter is transferred onto a Petri dish.
- Incubate and count colonies.

3. Most Probable Number (MPN):

- Used mainly to measure bacteria that will not grow on solid medium.
- Dilute a sample repeatedly and inoculate several broth tubes for each dilution point.
- Count the number of positive tubes in each set.
- Statistical method: Determines 95% probability that a bacterial population falls within a certain range.

4. Direct Microscopic Count:



- A specific volume of a bacterial suspension (0.01 ml) is placed on a microscope slide with a special grid.
- Stain is added to visualize bacteria.
- Cells are counted and multiplied by a factor to obtain concentration.

Advantages:

- No incubation time required.

Disadvantages:

- Cannot always distinguish between live and dead bacteria.
- Motile bacteria are difficult to count.
- Requires a high concentration of bacteria (10 million/ml).

Indirect Methods of Measurement

1. Turbidity:

- As bacteria multiply in media, it becomes turbid.
- Use a spectrophotometer to determine % transmission or absorbance.
- Multiply by a factor to determine concentration.

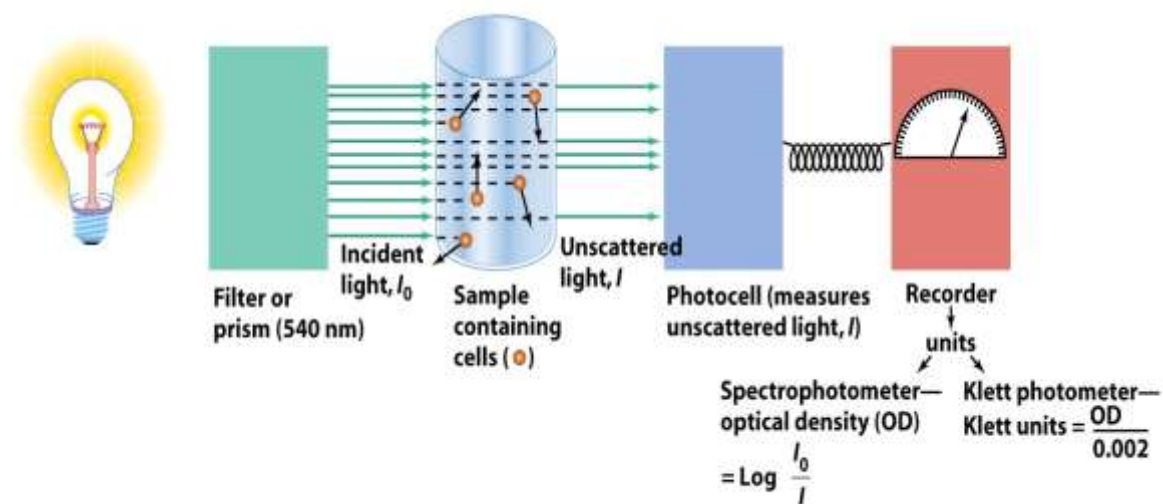
Advantages:

- No incubation time required.

Disadvantages:

- Cannot distinguish between live and dead bacteria.
- Requires a high concentration of bacteria (10 to 100 million cells/ml).

Spectrophotometer



2. Metabolic Activity:

- As bacteria multiply in media, they produce certain products:
 - Carbon dioxide
 - Acids
- Measure metabolic products.
- Expensive

3. Dry Weight:

- Bacteria or fungi in liquid media are centrifuged.
- Resulting cell pellet is weighed.
- Doesn't distinguish live and dead cells.

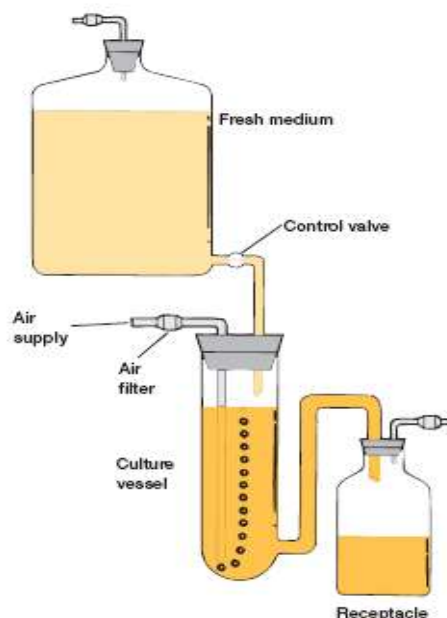
The Continuous Culture of Microorganisms

The growth of microorganisms in an open system, a system with constant environmental conditions maintained through continual provision of nutrients and removal of wastes. These conditions are met in the laboratory by a continuous culture system. A microbial population can be maintained in the exponential growth phase and at a constant biomass concentration for extended periods in a continuous culture system.

Two major types of continuous culture systems commonly are used: (1) chemostat and (2) turbidostat.

The Chemostat

A chemostat is constructed so that sterile medium is fed into the culture vessel at the same rate as the media containing microorganisms is removed.



The culture medium for a chemostat possesses an essential nutrient (e.g., an amino acid) in limiting quantities. Because of the presence of a limiting nutrient, the growth rate is determined by the rate at which new medium is fed into the growth chamber, and the final cell density depends on the concentration of the limiting nutrient. The rate of nutrient exchange is expressed as the dilution rate (D), the rate at which medium flows through the culture vessel relative to the vessel volume, where f is the flow rate (ml/hr) and V is the vessel volume (ml).

$$D = f/V$$

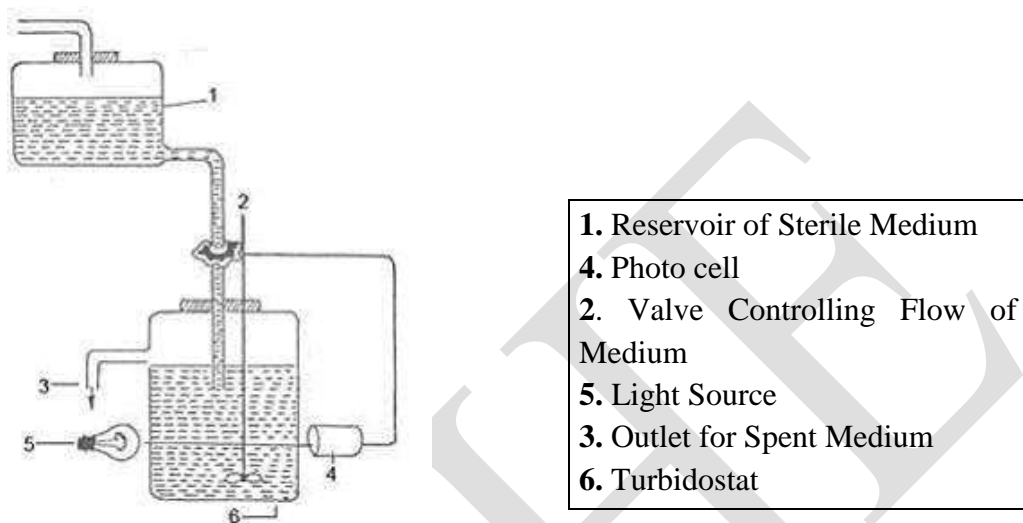
For example, if f is 30 ml/hr and V is 100 ml, the dilution rate is 0.30 hr^{-1} . Both the microbial population level and the generation time are related to the dilution rate.

The Turbidostat

The second type of continuous culture system, the turbidostat, has a photocell that measures the absorbance or turbidity of the culture in the growth vessel. The turbidostat differs from the chemostat in several ways. The dilution rate in a turbidostat varies rather than remaining constant, and its culture medium lacks a limiting nutrient. The turbidostat operates best at high dilution rates; the chemostat is most stable and effective at lower dilution rates.

Continuous culture systems are very useful because they provide a constant supply of cells in exponential phase and growing at a known rate. They make possible the study of microbial

growth at very low nutrient levels, concentrations close to those present in natural environments. These systems are essential for research in many areas for example, in studies on interactions between microbial species under environmental conditions resembling those in a freshwater lake or pond. Continuous systems also are used in food and industrial microbiology.

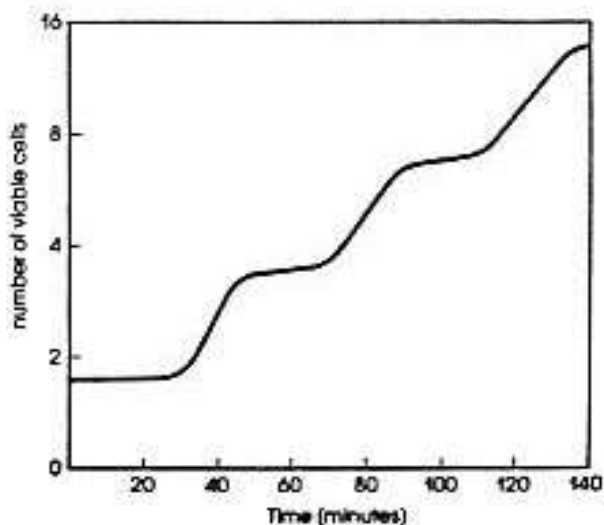


Turbidostat

Synchronous Growth of Bacteria

Studying the growth of bacterial populations in batch or continuous cultures does not permit any conclusions about the growth behavior of individual cells, because the distribution of cell size (and hence cell age) among the members of the population is completely random. Information about the growth behavior of individual bacteria can, however, be obtained by the study of synchronous cultures. Synchronized cultures must be composed of cells which are all at the same stage of the bacterial cell cycle. Measurements made on synchronized cultures are equivalent to measurements made on individual cells. A number of clever techniques have been devised to obtain bacterial populations at the same stage in the cell cycle. Some techniques involve manipulation of environmental parameters which induces the population to start or stop growth at the same point in the cell cycle, while others are physical methods for selection of cells that have just completed the process of binary fission. Theoretically, the smallest cells in a bacterial population are those that have just completed the process of cell division. Synchronous cultures

rapidly lose synchrony because not all cells in the population divide at exactly the same size, age or time.



Synchronous growth of a bacterial population

By careful selection of cells that have just divided, a bacterial population can be synchronized in the bacterial cell division cycle. Synchrony can be maintained for only a few generations

Diauxic growth

Diauxic growth is any cell growth characterized by cellular growth in two phases and can be illustrated with a diauxic growth curve. Diauxic growth, meaning double growth, is caused by the presence of two sugars on a culture growth media, one of which is easier for the target first, which leads to rapid growth followed by a lag phase. During the lag phase the cellular machinery used to metabolize the second sugar is activated and subsequently the second sugar is metabolized, example is *E. coli*. The bacterium is grown on a growth media containing two types of sugars, one of which is easier to metabolize than the other (for example glucose and lactose). First the bacterium will metabolize all the glucose and grow at a higher speed. Eventually, when all the glucose has been consumed, the bacterium will begin the process of expressing the genes to metabolize the lactose. This will only occur when all glucose in the media has been consumed. For these reasons, diauxic growth occurs in multiple phases.

Factors affecting microbial growth

Growth may be profoundly affected by a number of physical factors.

Temperature

Microorganisms as a group are able to grow over a wide range of temperatures, from around freezing to above boiling point. For any organism, the minimum and maximum growth temperatures define the range over which growth is possible; this is typically about 25–30 °C. Growth is slower at low temperatures because enzymes work less efficiently and also because

lipids tend to harden and there is a loss of membrane fluidity. Growth rates increase with temperature until the optimum temperature is reached and then the rate falls again. The optimum and limiting temperatures for an organism are a reflection of the temperature range of its enzyme systems, which in turn are determined by their three-dimensional protein structures. The optimum temperature is generally closer to the maximum growth temperature than the minimum. Once the optimum value is passed, the loss of activity caused by denaturation of enzymes causes the rate of growth to fall away sharply.

Psychrophiles occupy the other extreme of the temperature range; they can grow at 0°C, with optimal growth occurring at 15 °C or below. Such organisms are not able to grow at temperatures above 25 °C or so. Psychrotrophs, on the other hand, although they can also grow at 0 °C, have much higher temperature optima (20–30 °C). Members of this group are often economically significant due to their ability to grow on refrigerated foodstuffs. In the laboratory, appropriate temperatures for growth are provided by culturing in an appropriate incubator. These come in a variety of shapes and sizes, but all are thermostatically controlled and generally hold the temperature within a degree or two of the desired value.

Mesophiles

The majority of microorganisms achieve optimal growth at 'middling' temperatures of around 20–45 °C; these are called mesophiles.

Thermophiles

Thermophiles are organisms which grow at much higher temperatures. Typically, these would be capable of growth within a range of about 40–80 °C, with an optimum around 50–65 °C. The growth range of many thermophiles extends into the mesophilic regions, these species are termed as facultative thermophiles. Other thermophiles that cannot grow in the mesophilic range are termed as true thermophiles, obligate thermophiles or steno thermophiles.

Extreme thermophiles have optimum values in excess of this, and can tolerate temperatures in excess of 100°C.

pH

Microorganisms are strongly influenced by the prevailing pH of their surroundings. As with temperature, we can define minimum, optimum and maximum values for growth of a particular type. The pH range between minimum and maximum values is greater in fungi than it is in bacteria. Most microorganisms grow best around neutrality (pH 7). Many bacteria prefer slightly alkaline conditions but relatively few are tolerant of acid conditions, and fewer still are acidophilic.

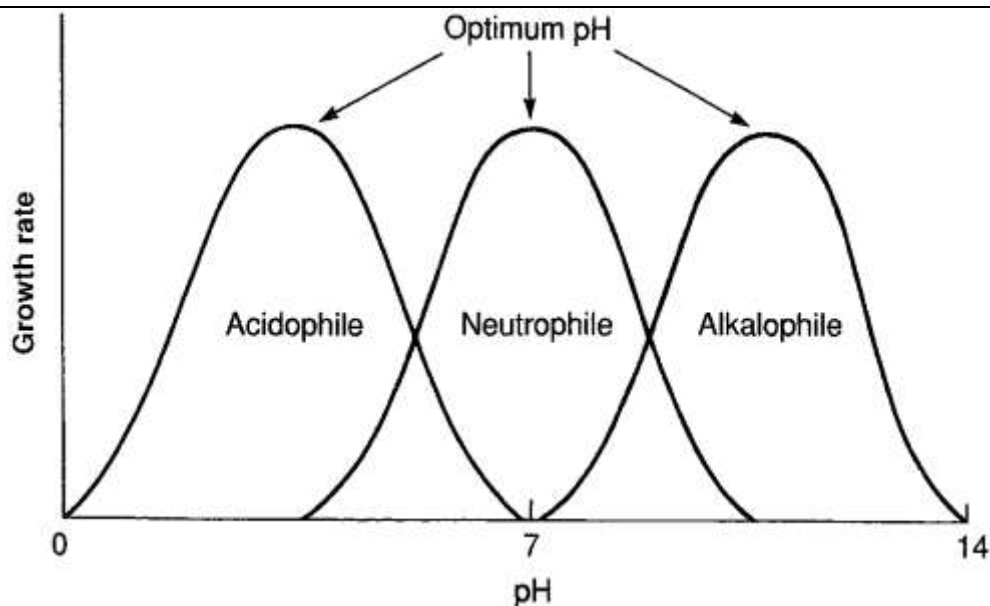


Figure Effect of pH on microbial growth rate. Individual species of microorganism occupy a relatively narrow range of pH.

Although for most species this is around neutrality, both acidophilic and alkalophilic forms exist. The shape of the curve reflects the properties of a particular organism's enzymes and other proteins. Fungi, on the other hand, generally prefer slightly acid conditions and therefore tend to dominate bacteria when these prevail. The reason for the growth rate falling away either side of the optimum value is again due to alterations in three-dimensional protein structure. The pH value of growth media is adjusted to the desired value by the addition of acid or alkali during its preparation. The metabolic activities of microorganisms often means that they change the pH of their environment as growth proceeds, so it is important in a laboratory growth medium that a desirable pH is not only set but maintained. This is achieved by the use of an appropriate buffer system. Phosphate buffers are widely used in the microbiology laboratory; they enable media to minimize changes in their pH when acid or alkali is produced

Gaseous requirement

The principal gases that affect bacterial growth are oxygen and carbondioxide. Bacteria display such a wide variety of responses to free oxygen that it is convenient to divide them into four groups on the following bases:

Aerobic bacteria

Aerobic bacteria require oxygen for growth and can grow when incubated in an air atmosphere. (ie., 21 percent oxygen).

Anaerobic bacteria

Anaerobic bacteria do not use oxygen to obtain energy, moreover, oxygen is toxic for them and they cannot grow when incubated in an air atmosphere. Some can tolerate low levels of oxygen (nonstringent or tolerant anaerobes), but others (stringent or strict anaerobes) cannot tolerate even low levels and may die upon brief exposure to air.

Facultatively anaerobic bacteria

Facultatively anaerobic bacteria do not require oxygen for growth, although they may use it for energy production if it is available. They are not inhibited by oxygen and usually grow as well under an air atmosphere as they do in the absence of oxygen.

Microaerophilic bacteria

Microaerophilic bacteria require low levels of oxygen for growth but cannot tolerate the level of oxygen present in an air atmosphere.

Carbon dioxide

Heterotrophic bacteria also require small amounts of carbon dioxide, which is incorporated into various metabolic intermediates. This dependency can be demonstrated by the failure of these organisms to grow if carbon dioxide is deliberately removed from the atmosphere. Microorganisms have different oxygen requirements. In a static culture, microorganisms occupy different regions of the medium, reflecting their pattern of oxygen usage. (a) Obligate aerobes must grow at or near the surface, where oxygen is able to diffuse. (b) Facultative anaerobes are able to adjust their metabolism to the prevailing oxygen conditions. (c) Obligate anaerobes, in contrast, occupy those zones where no oxygen is present at all. (d) Aerotolerant anaerobes do not use oxygen, but neither are they inhibited by it. (e) Microaerophiles have specific oxygen requirements, and can only grow within a narrow range of oxygen tensions

Osmotic pressure

Osmosis is the diffusion of water across a semipermeable membrane from a less concentrated solution to a more concentrated one, equalising concentrations. The pressure required to make this happen is called the osmotic pressure. If a cell was placed in a hypertonic solution (one whose solute concentration is higher) osmosis would lead to a loss of water from the cell (plasmolysis). This is the basis of using high concentrations of salt or other solutes in preserving foods against microbial attack. In the opposite situation, water would pass from a dilute (hypotonic) solution into the cell, causing it to swell and burst. The rigid cell walls of bacteria prevent them from bursting; this, together with their minute size, makes them less sensitive to variations in osmotic pressure than other types of cell. They are generally able to tolerate NaCl concentrations of between 0.5 and 3.0 per cent. Haloduric ('salt-tolerant') bacteria are able to tolerate concentrations ten times as high, but prefer lower concentrations, whereas halophilic ('salt-loving') forms are adapted to grow best in conditions of high salinity such as those that prevail in the Dead Sea in the Middle East. In order to do this without plasmolysis occurring, they must build up a higher internal solute concentration, which they do by actively concentrating potassium ions inside the cell.

Light

Phototrophic organisms require light in order to carry out photosynthesis. In the laboratory, care must be taken that light of the correct wavelength is used, and that the source used does not also act as a heat source. Fluorescent light produces little heat, but does not provide the wavelengths in excess of 750 nm needed by purple and green photosynthetic bacteria.

Unit – II

Possible Questions

Two Marks

1. Define growth.
2. What are coenocytic?
3. What is batch culture?
4. What is exponential phase?
5. What are different phases of growth curve?
6. What are the disadvantages of pour plate technique?
7. Mention the direct and indirect methods of measurement of microbial growth.
8. Write the advantages and disadvantages of direct microscope count.
9. How will you classify microorganisms based on temperature?
10. What are the two types of continuous culture of microorganisms?
11. Write the difference between turbidostat and chemostat.
12. What are the factors affecting the growth of microorganisms?
13. What is lag phase?
14. How will you classify microorganisms based on oxygen?

Eight Marks

1. Define growth and explain the different phases of microbial growth with proper example
2. Give a detail account on Synchronous culture, diauxic culture and its application.
3. Explain in detail on the factors influencing the microbial growth.
4. Explain the direct and indirect methods used for the measurement of microbial growth.
5. Give a detailed account on the environmental factors (Oxygen and temperature) on growth of microbial growth.
6. Explain the methods that are used for the measurement of microbial culture.
7. Explain the turbidimetric method used for the measurement of microbial growth.

UNIT II

Microbial population can be maintained in a state of exponential growth over a long
Which of the following is used for continuous culture ?

At which of the following temperature is the growth rate greatest ?

A bacterial culture began with cells and ended with 128 cells how many generations

Microbial cultures composed of cells that are all the same stage of the cell cycle are c

Microbial population can be maintained in a state of exponential growth over a long

The time required for the doubling of cell mass is known as _____

The formula for calculating total number of bacterial cell after 'n' number of generat

The time required for the doubling of cell mass is known as _____

Growth rate is the reciprocal of _____

In _____ phase rate of multiplication of bacteria increases with time.

If a cell concentration is periodically measured in a fresh medium _____ de

The log growth phase is also called as _____.

Too much of acid or base _____

The growth is modified by controlling and monitoring the turbidity of the culture is c

A few organisms can reduce elemental nitrogen to ammonia and this process of nitro

The _____ of the microorganism is the time that it takes for the cell to repr

Microbial growth increases the number of cells and the _____.

Reproduction of bacterial cells take place by _____.

Doubling time is otherwise called as _____.

The generation time is as short as 20 minutes under optimal condition in _____.

Methanococcus has a doubling time of _____ minutes

_____ are responsible for the biosynthesis of proteins

In the atmosphere the availability of water is expressed as _____.

The typical growth curve of a bacterial culture begins with the _____.

The growth phase at which there is no net increase in bacterial cell numbers is called

The number of viable cells beginning to decline signally the onset of the _____

_____ measures the turbidity of the culture in the growth vessel.

The growth rate of bacteria in nature can be estimated by using _____.

_____ of bacteria occurs when all cells divide at the same time.

Some bacteria known as _____ grow only at temperature near their opti

The alkaliphiles occupy the _____

Proton motive force used to synthesis the _____

Microorganisms which can thrive and grow in harsh conditions are often called _____

_____ is a hydrogen ion activity

Extreme alkalophiles maintain their internal pH closer to _____

Which of the following media would not be used to culture aerobes?

Which one of the following temperature would most likely kill a mesophiles?

The term trace elements refers to _____

Diauxic growth by utilizing _____ in growth media

High pressure denature the _____ in vegetative cell

Endospores can resist _____

Microorganism in high concentration of salts and sugars undergo _____

The effect of radiation depend on _____

_____ is a nonionizing radiation

The **growth** curve generated by an organism which has two **growth** peaks

Diauxic growth _____

Synchronous culture _____

Synchronous culture _____

Growth occurs at a constant specific growth rate

Turbidostat _____

Petroff Hausser counting chamber _____

Measurement of bacterial cel directly

McFarland standards are used as a reference to adjust the _____

Membrane filter technique

_____ can survive in all sorts of extremely hostile environment.

_____ Can survive in hot spring

Thermus thermophilus is example for _____

Organisms that live in environment with very high concentration of salt

Time between two consecutive generation is _____

ACADEMY OF HIGHER EDUCATION
 DEPARTMENT OF MICROBIOLOGY
 BIOLOGY AND METABOLISM (17MBU202)

OPTION A	OPTION B	OPTION C	OPTION D
Batch culture	Continuous culture	Synchronous culture	Pure culture
Role tube	Chemostat	Thermostat	Conical flask
Optimum	Maximum	Minimum	Cardinal
64	32	6	5
Axenic culture	Pure culture	Mixed culture	Synchronous culture
Batch culture	Continuous culture	Synchronous culture	Pure culture
Doubling time	Generation time	Generation gap	Developing time
$N = 1 \times 2^n$	$N = 2 \times 2^n$	$N = 1 \times 1^n$	$N = 2 \times 1^n$
Doubling time	Generation time	Generation gap	Developing time
Doubling time	Cell division	Binary fission	Generation time
Lag	Log	Stationary	Decline
Growth curve	Growth rate	Generation time	Generation period
Stationary phase	Exponential growth	Lag phase	Death phase
promote cellular activity	disturb the cellular	enhance the cell gro	activate the metabolism
Synchronous method	Batch culture meth	Turbidostat	Thermostat
Biological nitrogen fixati	Biological nitrite fi	Biological ammonia	Ammonification
Growth curve	Growth amount	Growth rate	Biomass
Biomass	Rate	Doubling time	Structure
Pollination	Binary fission	Mitosis	Meiosis
Growth curve	Growth rate	Generation time	Generation period
Klebsiella	Proteus	Salmonella	E. coli
20	10	30	50
Nucleus	Ribosome	DNA	Sulphur
Relative humidity	Xerotolerant	Osmosis	Water activity
Log phase	Lag phase	Death phase	Exponential growth phase
Stationary phase	Exponential growth	Lag phase	Death phase
Log phase	Lag phase	Death phase	Exponential growth phase
Synchronous method	Batch culture meth	Turbidostat	Thermostat
Synchronous method	Batch culture meth	Turbidostat	Chemostat
Synchronous culture	Batch culture meth	Turbidostat culture	Thermostat culture
Euthermal	Stenothermal	thermophiles	Capnophiles
High end of pH spectrum	Low end of pH spe	neare to neutral Ph	neutral Ph
ADP	NADH	NADPH ₂	ATP
Halophiles	Halophiles	Extremophiles	Mesophiles
Temperature	aeration	osmotic pressure	Ph
alkalinity	acidity	high alkalinity	neutrality
selective media	differential media	complex media	reducing media
50	9	60	37

thye elements CHONPS	vitamines	nitogen, phosphorou	small mineral requirements
two sugar	single sugar	aminoacid	vitamine
lipid	cell wall	protein	membrane
low salinity	salinity	water activity	Desiccation
damage	osmosis	cell lysis	plasmolysis
speed	intensity	dose	exposure
electron beam	UV	Gamma	X ray
Diauxic growth	generation time	continuous growth	batch culture
single growth	double growth	triple growth	slow growth
further no growth	cell divide slowly	All cell divide simul	fast cell growth
all cell same age	fast cell growth	further no growth	cell divide slowly
<u>chemostat</u>	<u>Batch culture meth</u>	<u>diauxic culture</u>	<u>log phase</u>
Batch culture	continuous culture	synchronous culture	Diauxic culture
measuring the cell	view the motility	view the shape	measure the size
Dry weight	turbidity	nitrogen content	Petroff Hausser counting chamb
Growth curve	turbidity of bacteri	medium	dilution
measure the bacterial cell	filtration of all mic	purification	killing the microbes
mesophiles	thermophiles	Extremophiles	Halophiles
Themophiles	mesophiles	Extremophiles	Halophiles
Themophiles	mesophiles	Extremophiles	Halophiles
mesophiles	thermophiles	Extremophiles	Halophiles
Lag phase	log phase	decline	generation time

ANSWER KEY

Continuous culture

Conical flask

Optimum

6

Synchronous culture

Continuous culture

Doubling time

$N = 1 \times 2^n$

Doubling time

Cell division

Log

Growth curve

Exponential growth phase

disturb the cellular activity

Turbidostat

Biological nitrite fixation

Growth rate

Rate

Binary fission

Generation time

E. coli

20

Ribosome

Relative humidity

Lag phase

Stationary phase

Death phase

Turbidostat

Chemostat

Synchronous culture

Stenothermal

High end of pH spectrum

ATP

Extremophiles

Ph

neutrality

reducing media

60

small mineral requirements

two sugar

protein

Desiccation

plasmolysis

intensity

UV

Diauxic growth

double growth

All cell divide simultaneously

all cell same age

chemostat

continuous culture

measuring the cell

er Petroff Hausser counting chamber

turbidity of bacterial suspensions

measure the bacterial cell

Extremophiles

Thermophiles

Thermophiles

Halophiles

generation time

UNIT – 3**CARBOHYDRATE METABOLISM**

Metabolism may be divided into two major parts. In **catabolism** [Greek *cata*, down, and *ballein*, to throw] larger and more complex molecules are broken down into smaller, simpler molecules with the release of energy. Some of this energy is trapped and made available for work; the remainder is released as heat. The trapped energy can then be used in anabolism, the second area of metabolism.

Anabolism [Greek *ana*, up] is the synthesis of complex molecules from simpler ones with the input of energy. An anabolic process uses energy to increase the order of a system. Although the division of metabolism into two major parts is convenient and commonly employed, not all energy-yielding processes are comfortably encompassed by the previous definition of catabolism unless it is expanded to include processes that do not involve the degradation of complex organic molecules. In a broader sense, microorganisms usually use one of three sources of energy. Phototrophs capture radiant energy from the sun. Chemoorganotrophs oxidize organic molecules to liberate energy, while chemolithotrophs employ inorganic nutrients as energy sources.

Carbohydrates and other nutrients serve two functions in the metabolism of heterotrophic microorganisms: (1) they are oxidized to release energy, and (2) they supply carbon or building blocks for the synthesis of new cell constituents. Although many anabolic pathways are separate from catabolic routes, there are **amphibolic pathways** [Greek *amphi*, on both sides] that function both catabolically and anabolically. Two of the most important are the glycolytic pathway and the tricarboxylic acid cycle. Most reactions in these two pathways are freely reversible and can be used to synthesize and degrade molecules. The few irreversible catabolic steps are bypassed in biosynthesis with special enzymes that catalyze the reverse reaction. For example, the enzyme fructose biphosphatase reverses the phosphofructokinase step when glucose is synthesized from pyruvate. The presence of two separate enzymes, one catalyzing the reversal of the other's reaction, permits independent regulation of the catabolic and anabolic functions of these amphibolic pathways.

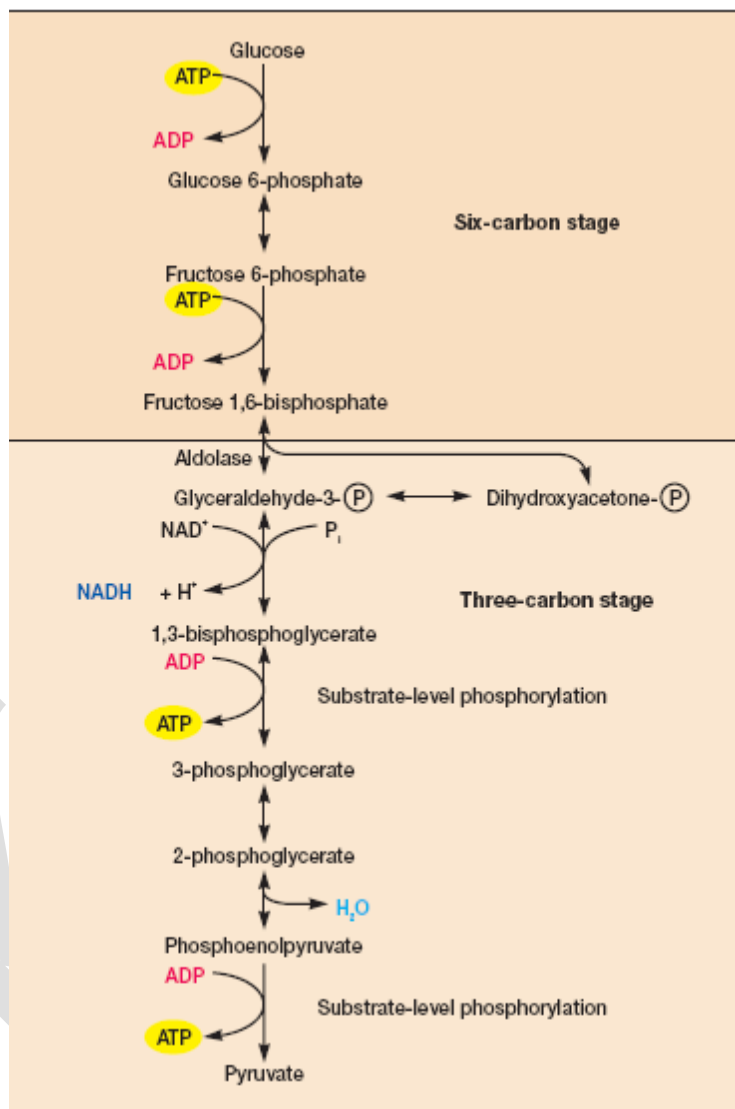
The Breakdown of Glucose to Pyruvate

Microorganisms employ several metabolic pathways to catabolize glucose and other sugars. Because of this metabolic diversity, their metabolism is often confusing. To avoid confusion as much as possible, the ways in which microorganisms degrade sugars to pyruvate and similar intermediates are introduced by focusing on only three routes: (1) glycolysis, (2) the pentose phosphate pathway, and (3) the Entner-Doudoroff pathway.

The Glycolytic Pathway

The **Embden-Meyerhof** or **glycolytic pathway** is undoubtedly the most common pathway for glucose degradation to pyruvate in stage two of catabolism. It is found in all major groups of

microorganisms and functions in the presence or absence of O₂. **Glycolysis** [Greek *glyco*, sweet, and *lysis*, a loosening] is located in the cytoplasmic matrix of procaryotes and eucaryotes. The pathway as a whole may be divided into two parts



Glycolysis - The glycolytic pathway for the breakdown of glucose to pyruvate. The two stages of the pathway and their products are indicated.

In the initial six-carbon stage, glucose is phosphorylated twice and eventually converted to fructose 1, 6- bisphosphate. Other sugars are often fed into the pathway by conversion to glucose

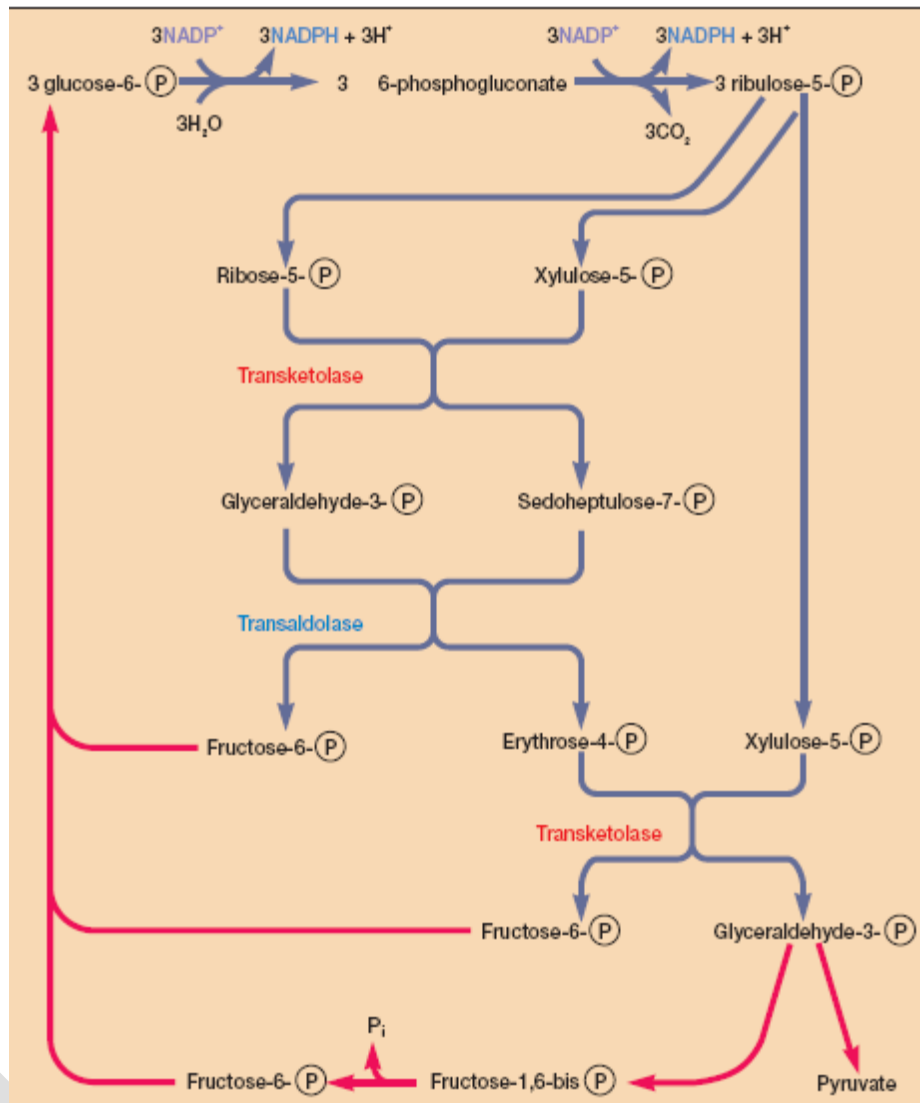
6-phosphate or fructose 6-phosphate. This preliminary stage does not yield energy; in fact, two ATP molecules are expended for each glucose. These initial steps “prime the pump” by adding phosphates to each end of the sugar. The phosphates will soon be used to make ATP. The three-carbon stage of glycolysis begins when the enzyme fructose 1,6-bisphosphate aldolase catalyzes the cleavage of fructose 1,6-bisphosphate into two halves, each with a phosphate group. One of the products, glyceraldehyde 3-phosphate, is converted directly to pyruvate in a five-step process. Because the other product, dihydroxyacetone phosphate, can be easily changed to glyceraldehyde 3-phosphate, both halves of fructose 1,6-bisphosphate are used in the three-carbon stage. Glyceraldehyde 3-phosphate is first oxidized with NAD⁺ as the electron acceptor, and a phosphate is simultaneously incorporated to give a high-energy molecule called 1,3-bisphosphoglycerate. The high energy phosphate on carbon one is subsequently donated to ADP to produce ATP. This synthesis of ATP is called **substrate-level phosphorylation** because ADP phosphorylation is coupled with the exergonic breakdown of a high-energy substrate molecule. A somewhat similar process generates a second ATP by substrate-level phosphorylation. The phosphate group on 3-phosphoglycerate shifts to carbon two, and 2-phosphoglycerate is dehydrated to form a second high-energy molecule, phosphoenolpyruvate. This molecule donates its phosphate to ADP forming a second ATP and pyruvate, the final product of the pathway.

The glycolytic pathway degrades one glucose to two pyruvates by the sequence of reactions just outlined. ATP and NADH are also produced. The yields of ATP and NADH may be calculated by considering the two stages separately. In the six-carbon stage two ATPs are used to form fructose 1,6-bisphosphate. For each glyceraldehydes 3-phosphate transformed into pyruvate, one NADH and two ATPs are formed. Because two glyceraldehyde 3-phosphates arise from a single glucose (one by way of dihydroxyacetone phosphate), the three-carbon stage generates four ATPs and two NADHs per glucose. Subtraction of the ATP used in the six-carbon stage from that produced in the three-carbon stage gives a net yield of two ATPs per glucose. Thus the catabolism of glucose to pyruvate in glycolysis can be represented by the following simple equation.



The Pentose Phosphate Pathway

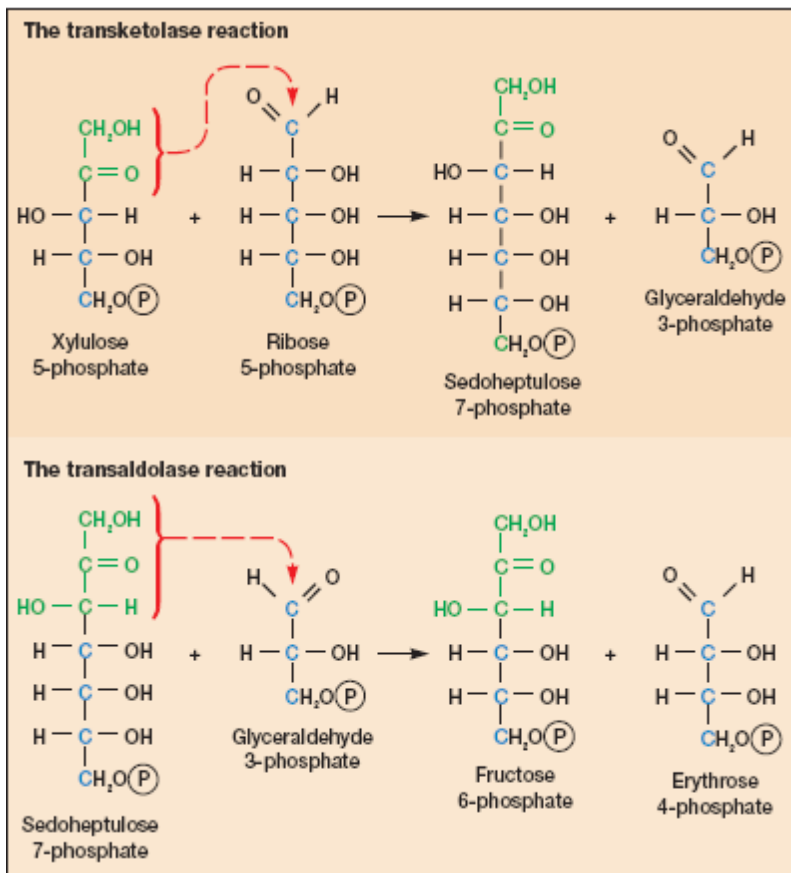
A second pathway, the **pentose phosphate** or **hexose monophosphate pathway** may be used at the same time as the glycolytic pathway or the Entner-Doudoroff sequence. It can operate either aerobically or anaerobically and is important in biosynthesis as well as in catabolism. The pentose phosphate pathway begins with the oxidation of glucose 6-phosphate to 6-phosphogluconate followed by the oxidation of 6-phosphogluconate to the pentose ribulose 5-phosphate and CO₂.



The Pentose Phosphate Pathway

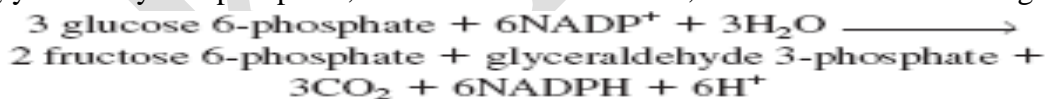
The conversion of three glucose 6-phosphate molecules to two fructose 6-phosphates and a glyceraldehyde 3-phosphate is traced. The fructose 6-phosphates are changed back to glucose 6-phosphate. The glyceraldehyde 3-phosphate can be converted to pyruvate or combined with a molecule of dihydroxyacetone phosphate (from the glyceraldehyde 3-phosphate formed by a second turn of the pathway) to yield fructose 6-phosphate.

NADPH is produced during these oxidations. Ribulose 5-phosphate is then converted to a mixture of three- through seven-carbon sugar phosphates. Two enzymes unique to this pathway play a central role in these transformations: (1) transketolase catalyzes the transfer of two-carbon ketol groups, and (2) transaldolase transfers a three-carbon group from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate.

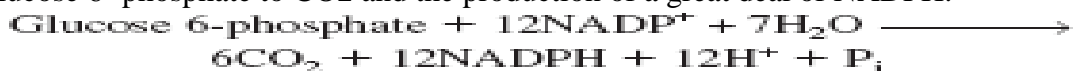


Transketolase and Transaldolase. Examples of the transketolase and transaldolase reactions of the pentose phosphate pathway. The groups transferred in these reactions are in color.

The overall result is that three glucose 6-phosphates are converted to two fructose 6-phosphates, glyceraldehyde 3-phosphate, and three CO₂ molecules, as shown in the following equation.



These intermediates are used in two ways. The fructose 6-phosphate can be changed back to glucose 6-phosphate while glyceraldehyde 3-phosphate is converted to pyruvate by glycolytic enzymes. The glyceraldehyde 3-phosphate also may be returned to the pentose phosphate pathway through glucose 6-phosphate formation. This results in the complete degradation of glucose 6-phosphate to CO₂ and the production of a great deal of NADPH.

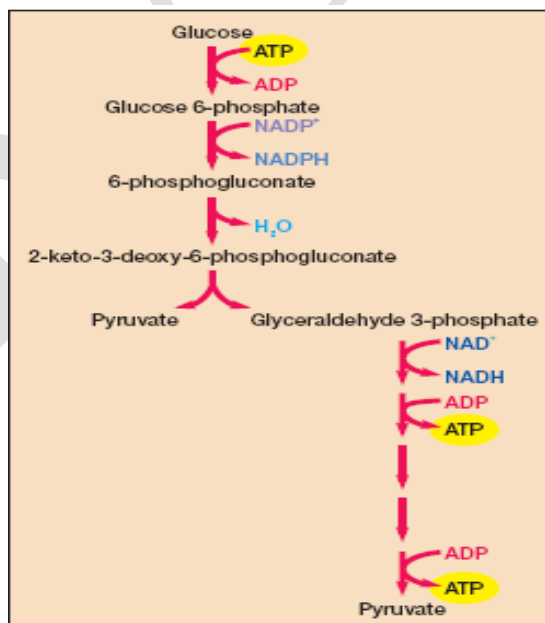


The pentose phosphate pathway has several catabolic and anabolic functions that are summarized as follows:

1. NADPH from the pentose phosphate pathway serves as a source of electrons for the reduction of molecules during biosynthesis.
2. The pathway synthesizes four- and five-carbon sugars for a variety of purposes. The four-carbon sugar erythrose 4-phosphate is used to synthesize aromatic amino acids and vitamin B6 (pyridoxal). The pentose ribose 5-phosphate is a major component of nucleic acids, and ribulose 1,5-bisphosphate is the primary CO₂ acceptor in photosynthesis. Note that when a microorganism is growing on a pentose carbon source, the pathway also can supply carbon for hexose production (e.g., glucose is needed for peptidoglycan synthesis).
3. Intermediates in the pentose phosphate pathway may be used to produce ATP. Glyceraldehyde 3-phosphate from the pathway can enter the three-carbon stage of the glycolytic pathway and be converted to ATP and pyruvate. The latter may be oxidized in the tricarboxylic acid cycle to provide more energy. In addition, some NADPH can be converted to NADH, which yields ATP when it is oxidized by the electron transport chain. Because five-carbon sugars are intermediates in the pathway, the pentose phosphate pathway can be used to catabolize pentoses as well as hexoses. Although the pentose phosphate pathway may be a source of energy in many microorganisms, it is more often of greater importance in biosynthesis. Several functions of the pentose phosphate pathway are mentioned again in chapter 10 when biosynthesis is considered more directly.

The Entner-Doudoroff Pathway

Although the glycolytic pathway is the most common route for the conversion of hexoses to pyruvate, another pathway with a similar role has been discovered. The **Entner-Doudoroff pathway** begins with the same reactions as the pentose phosphate pathway, the formation of glucose 6-phosphate and 6-phosphogluconate.

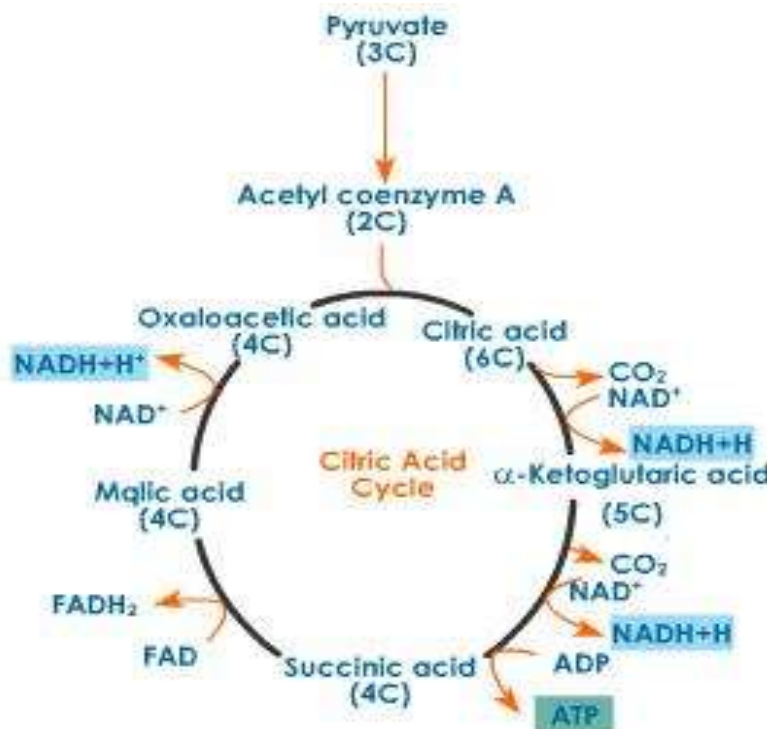


The Entner-Doudoroff Pathway.

Instead of being further oxidized, 6- phosphogluconate is dehydrated to form 2-keto-3-deoxy-6-phosphogluconate or KDPG, the key intermediate in this pathway. KDPG is then cleaved by KDPG aldolase to pyruvate and glyceraldehydes 3-phosphate. The glyceraldehyde 3-phosphate is converted to pyruvate in the bottom portion of the glycolytic pathway. If the Entner-Doudoroff pathway degrades glucose to pyruvate in this way, it yields one ATP, one NADPH, and one NADH per glucose metabolized. Most bacteria have the glycolytic and pentose phosphate pathways, but some substitute the Entner-Doudoroff pathway for glycolysis. The Entner-Doudoroff pathway is generally found in *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Agrobacterium*, and a few other gram-negative genera. Very few gram-positive bacteria have this pathway, with *Enterococcus faecalis* being a rare exception.

The Tricarboxylic Acid Cycle

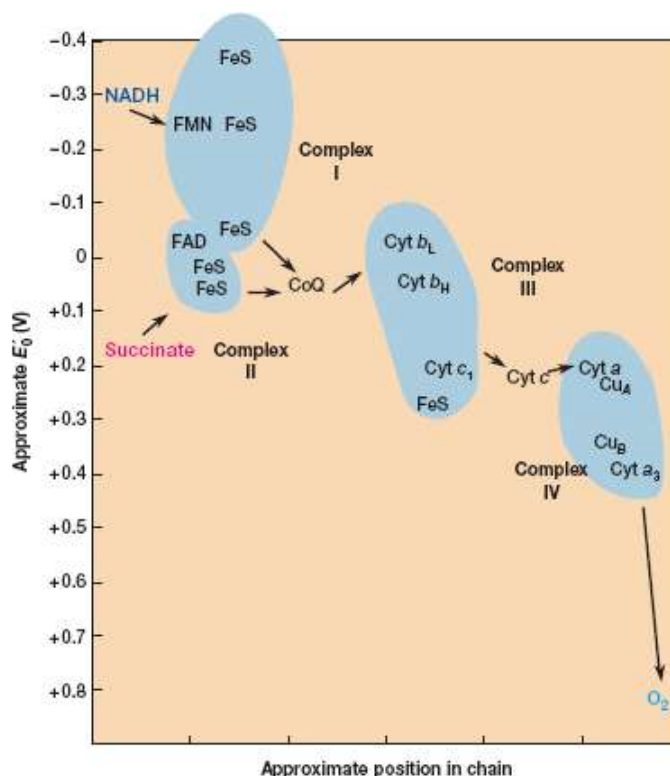
Although some energy is obtained from the breakdown of glucose to pyruvate by the pathways previously described, much more is released when pyruvate is degraded aerobically to CO₂ in stage three of catabolism. The multienzyme system called the pyruvate dehydrogenase complex first oxidizes pyruvate to form CO₂ and acetyl coenzyme A (acetyl-CoA), an energy-rich molecule composed of coenzyme A and acetic acid joined by a high energy thiol ester bond. Acetyl-CoA arises from the catabolism of many carbohydrates, lipids, and amino acids. It can be further degraded in the tricarboxylic acid cycle. The substrate for the tricarboxylic acid (TCA) cycle, citric acid cycle, or Krebs cycle is acetyl-CoA.



The Tricarboxylic Acid Cycle

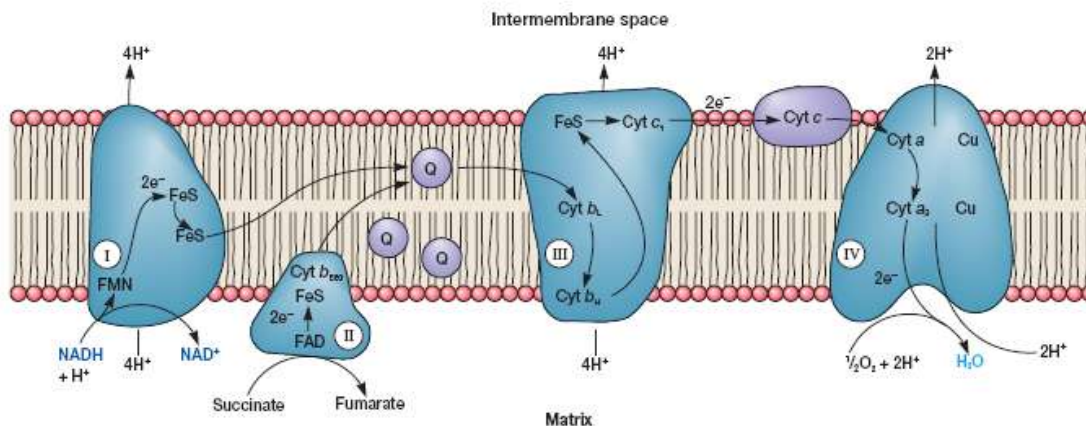
The traditional way to think about the cycle is in terms of its intermediates and products, and the chemistry involved in each step. In the first reaction acetyl-CoA is condensed with a four carbon intermediate, oxaloacetate, to form citrate and to begin the six-carbon stage. Citrate (a tertiary alcohol) is rearranged to give isocitrate, a more readily oxidized secondary alcohol. Isocitrate is subsequently oxidized and decarboxylated twice to yield α -ketoglutarate, then succinyl-CoA. At this point two NADHs are formed and two carbons are lost from the cycle as CO_2 . Because two carbons were added as acetyl-CoA at the start, balance is maintained and no net carbon is lost. The cycle now enters the four-carbon stage during which two oxidation steps yield one FADH_2 and one NADH per acetyl-CoA. In addition, GTP (a high-energy molecule equivalent to ATP) is produced from succinyl-CoA by substrate-level phosphorylation. Eventually oxaloacetate is reformed and ready to join with another acetyl-CoA. The TCA cycle generates two CO_2 s, three NADHs, one FADH_2 , and one GTP for each acetyl-CoA molecule oxidized.

Another way to think of the TCA cycle is in terms of its function as a pathway that oxidizes acetyl-CoA to CO_2 . From this perspective, the first step is the attachment of an acetyl group to the acetyl carrier, oxaloacetate, to form citrate. The second stage begins with citrate and ends in the formation of succinyl-CoA. Here, the acetyl carrier portion of citrate loses two carbons when it is oxidized to give two CO_2 s. The third and last stage converts succinyl-CoA back to oxaloacetate, the acetyl carrier, so that it can pick up another acetyl group. TCA cycle enzymes are widely distributed among microorganisms. The complete cycle appears to be functional in many aerobic bacteria, free-living protozoa, and most algae and fungi. This is not surprising

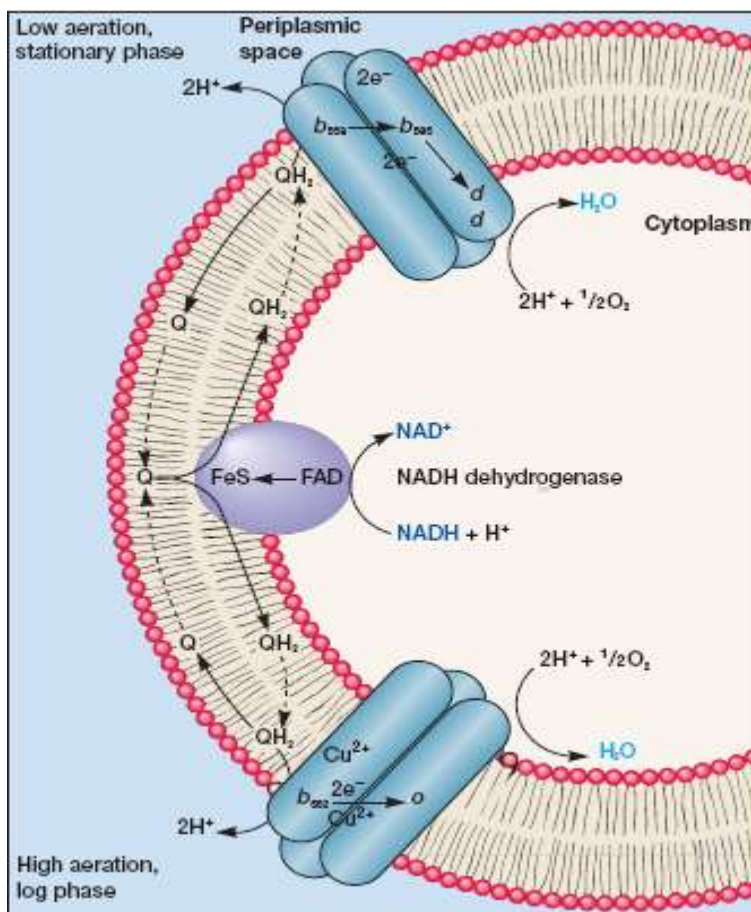


The Mitochondrial Electron Transport Chain

The electrons flow from carriers with more negative reduction potentials to those with more positive potentials and eventually combine with O_2 and H^+ to form water. The electrons move down this potential gradient much like water flowing down a series of rapids. The difference in reduction potentials between O_2 and NADH is large, about 1.14 volts, and makes possible the release of a great deal of energy. The potential changes at several points in the chain are large enough to provide sufficient energy for ATP production, much like the energy from waterfalls can be harnessed by waterwheels and used to generate electricity. The electron transport chain breaks up the large overall energy release into small steps. Some of the liberated energy is trapped in the form of ATP. As will be seen shortly, electron transport at these points may generate proton and electrical gradients. These gradients can then drive ATP synthesis. The electron transport chain carriers reside within the inner membrane of the mitochondrion or in the bacterial plasma membrane. The mitochondrial system is arranged into four complexes of carriers, each capable of transporting electrons part of the way to O_2 . Coenzyme Q and cytochrome *c* connect the complexes with each other.



The process by which energy from electron transport is used to make ATP is called oxidative phosphorylation. Thus as many as three ATP molecules may be synthesized from ADP and Pi when a pair of electrons passes from NADH to an atom of O₂. This is the same thing as saying that the phosphorus to oxygen (P/O) ratio is equal to 3. Because electrons from FADH₂ only pass two oxidative phosphorylation points, the maximum P/O ratio for FADH₂ is 2. The actual P/O ratios may be less than 3.0 and 2.0 in eucaryotic mitochondria. The preceding discussion has focused on the eucaryotic mitochondrial electron transport chain. Although some bacterial chains resemble the mitochondrial chain, they are frequently very different. They vary in their electron carriers (e.g., in their cytochromes) and may be extensively branched. Electrons often can enter at several points and leave through several terminal oxidases. Bacterial chains also may be shorter and have lower P/O ratios than mitochondrial transport chains. Thus procaryotic and eucaryotic electron transport chains differ in details of construction although they operate using the same fundamental principles. The electron transport chains of *Escherichia coli* and *Paracoccus denitrificans* will serve as examples of these differences.

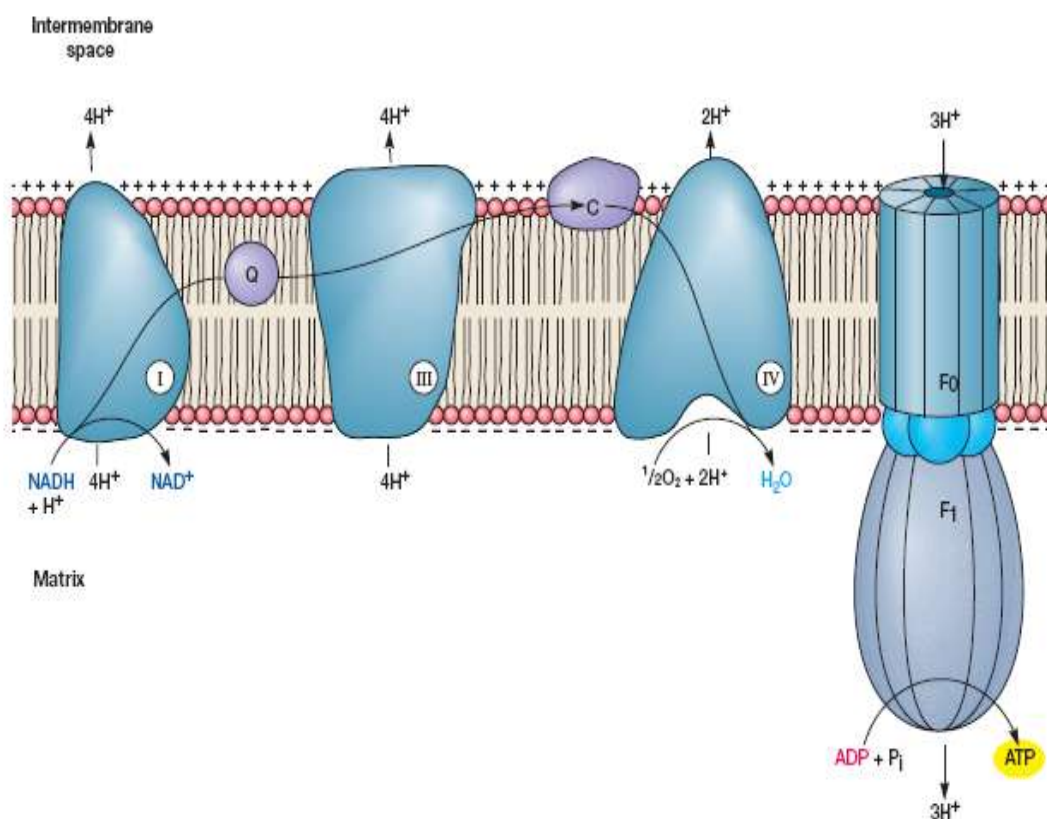


The Aerobic Respiratory System of *E. coli*

Although it transports electrons from NADH to acceptors and moves protons across the plasma membrane, the *E. coli* chain is quite different from the mitochondrial chain. For example, it is branched and contains a quite different array of cytochromes. Coenzyme Q or ubiquinol donates electrons to both branches, but they operate under different growth conditions. The cytochrome *d* branch has very high affinity for oxygen and functions at low oxygen levels. It is not as efficient as the cytochrome *o* branch because it does not actively pump protons. The cytochrome *o* branch has moderately high affinity for oxygen, is a proton pump, and operates at higher oxygen concentrations.

Oxidative Phosphorylation

The mechanism by which oxidative phosphorylation takes place has been studied intensively for years. Currently the most widely accepted hypothesis about how oxidative phosphorylation occurs is the chemiosmotic hypothesis. According to the chemiosmotic hypothesis, first formulated in 1961 by the British biochemist Peter Mitchell, the electron transport chain is organized so that protons move outward from the mitochondrial matrix and electrons are transported inward.



Chemiosmosis

Proton movement may result either from carrier loops, or from the action of special proton pumps that derive their energy from electron transport. The result is **proton motive force (PMF)**, composed of a gradient of protons and a membrane potential due to the unequal distribution of charges. When protons return to the mitochondrial matrix driven by the proton motive force, ATP is synthesized in a reversal of the ATP hydrolysis reaction. A similar process takes place in procaryotes, with electron flow causing the protons to move outward across the plasma membrane. ATP synthesis occurs when these protons diffuse back into the cell. The proton

motive force also may drive the transport of molecules across membranes and the rotation of bacterial flagella and thus plays a central role in procaryotic physiology.

The Yield of ATP in Glycolysis and Aerobic Respiration

The maximum ATP yield in eucaryotes from glycolysis, the TCA cycle, and electron transport can be readily calculated. The conversion of glucose to two pyruvate molecules during glycolysis gives a net gain of two ATPs and two NADHs. Because each NADH can yield a maximum of three ATPs during electron transport and oxidative phosphorylation (a P/O ratio of 3), the total aerobic yield from the glycolytic pathway is eight ATP molecules. Under anaerobic conditions, when the NADH is not oxidized by the electron transport chain, only two ATPs will be generated during the degradation of glucose to pyruvate.

When O₂ is present and the electron transport chain is operating, pyruvate is next oxidized to acetyl-CoA, the substrate for the TCA cycle. This reaction yields 2 NADHs because 2 pyruvates arise from a glucose; therefore 6 more ATPs are formed. Oxidation of each acetyl-CoA in the TCA cycle will yield 1 GTP (or ATP), 3 NADHs, and a single FADH₂ for a total of 2 GTPs (ATPs), 6 NADHs, and 2 FADH₂s from two acetyl-CoA molecules. As table 9.2 shows, this amounts to 24 ATPs when NADH and FADH₂ from the cycle are oxidized in the electron transport chain. Thus the aerobic oxidation of glucose to 6 CO₂ molecules supplies a maximum of 38 ATPs. In fact, the P/O ratios are more likely about 2.5 for NADH and 1.5 for FADH₂. Thus the total ATP aerobic yield from glucose may be closer to 30 ATPs rather than 38.

Unit – III
Possible Questions

Two Marks

1. What is catabolism?
2. Define anabolism.
3. Define fermentation.
4. What is respiration?
5. Mention the two amphibolic pathways.
6. What are exergonic and endergonic reactions?
7. What is substrate level phosphorylation?
8. What is energy and what kinds of work are carried out in a cell.
9. Name the Co-enzyme involved in the electron transport chain with any one function.
10. Define oxidative phosphorylation.
11. What are uncouplers? Give examples.
12. Give a neat sketch of electron transport chain of prokaryotes.

Eight Marks

1. Explain the concept of aerobic and anaerobic respiration.
2. Comment on the EMP pathway with energy calculations.
3. Explain oxidative phosphorylation & ATP generation.
4. Describe the characteristics of electron transport in bacteria.
5. Distinguish cyclic photophosphorylation from non- photophosphorylation?
6. Explain in detail about the Krebs's cycle.
7. Explain the ED pathway and its significance?
8. Comment on the mechanism of TCA cycle.
9. Explain the process of pentose phosphate pathway and its energy table.
10. Give an account on uncouplers and inhibitors.

UNIT III

The ED pathway is generally found in _____.

The number of ATP generated during ED pathway is _____.

The glycolytic pathway degrades one glucose to _____ pyruvates.

The pentose phosphate pathway is otherwise called as _____.

_____ enzymes catalyse the transfer of 2-C ketol groups in HMP pathway.

The EMP pathway occurs in the _____ of procaryotes and eucaryotes.

The process in which radiant energy is used to generate ATP is called _____.

The enzyme involved in the conversion of glucose to glucose-6 phosphate in EMP pathway is _____.

The conversion of fructose 6 phosphate to fructose 1,6-bisphosphate in EMP pathway is catalysed by _____ reaction.

The formation of ATP in EMP pathway is carried out by _____ reaction.

_____ enzyme of EMP pathway was lacking in ED pathway.

Glycolytic pathway generate _____ by substrate –level phosphorylation.

In Alcoholic fermentation, Pyruvate is converted to _____.

Purine base present in an ATP molecule is _____.

_____ is the most common pathway for glucose degradation to pyruvate.

The addition of phosphate group to a compound is called _____.

The compound that supplies electron for an electron-transport system is called _____.

In an electron transport chain _____ ions are pumped across the membrane and _____.

ATP is hydrolyzed to give _____.

Dissimilation is _____ of nutrients during which energy is released.

Glycolysis is dissimilatory pathway that results in the breakdown of a molecule of glucose.

Aerobic respiration the terminal electron receptor is _____.

Metabolism by glycolysis gives a net yield of _____ ATP molecules.

In comparing the efficiency of fermentation versus respiration with regard to ATP yield, _____.

Energy production in anaerobes is not by _____.

Sulphur is needed for the biosynthesis of aminoacids such as _____.

Which of the following statement is correct?

A molecule that loses a hydrogen atom is said to have been oxidized, because a hydroxyl group is removed.

Proton motive force can be used to synthesise _____.

During glycolysis which type of phosphorylation generates ATP?

Which of the following biochemical pathways occur only in microorganisms?

Fermentation yields _____ ATP per substrate molecule than respiration.

Which of the following require a high concentration of sodium?

The synthesis of ATP in fermentation is due to _____.

_____ pathway is used in homolactic acid fermentation.

The ethanol and carbon dioxide produced during heterolactic acid fermentation come from _____.

The process of break down a _____ is called glycolysis.

APS stands for _____.

The APS is phosphorylated by a second ATP molecule to form _____.

_____ serves as the precursor for thymidine triphosphate which occurs in DNA

The conversion of Glucose-6 phosphate to fructose 1,6 biphosphate catalysed by ____

All living organisms use _____ as central currency of energy.

In some of the metabolic pathway, called _____ substances are broken down into

_____ is a readily available intermediate of glycolysis.

Non cyclic photo phosphorylation _____

Molecular weight of ferridoxin is _____ dalton

_____ is an acidic protein

Glycolysis is dissimilatory pathway that results in the breakdown of a molecule of glu

Cyclic photophosphorylation _____

Photosystem I only reduce _____

The herbicide inhibits reduction of _____ activity in oxidative phospho

Precursor for purine biosynthesis _____

Nitrogen fixation reduces _____

Initial carrier which accepts electrons in electron transport chain is _____

_____ is needed to drive the formation of ATP.

The electrons are transferred unidirectionally in _____ pathway.

Electrons can flow cyclically in _____.

The photosystems has its own _____.

Photosystem I and II linked into unified pathway called _____.

Converts glucose into pyruvate

ACADEMY OF HIGHER EDUCATION
 DEPARTMENT OF MICROBIOLOGY
 BIOLOGY AND METABOLISM (17MBU202)

OPTION A	OPTION B	OPTION C	OPTION D
Pseudomonas	Streptococcus	Vibrio	Proteus
	2	1	3
	1	3	5
ED pathway	TCA cycle	HMP pathway	Krebs cycle
transaldolase	transketolase	epimerase	kinase
ribosomes	nucleus	plasma membrane	cytoplasmic matrix
Photophosphorylation	Oxidative phosphorylation	Substrate level phosphorylation	Fermentation
aldolase	Hexokinase	enolase	kinase
aldolase	Isomerase	Phosphofructokinase	Enolase
Aerobic respiration	Substrate level phosphorylation	Oxidative phosphorylation	Fermentation
6-phosphofructokinase	dehydrogenase	kinase	lyase
ATP	ADP	UTP	UDP
Ethanol & O ₂	Ethanol & CO ₂	Methanol & CO ₂	Methanol & O ₂
Cytosine	Threonine	Quanine	Adenine
HMP pathway	ED pathway	EMP pathway	TCA cycle
Carboxylation	Hydroxylation	Phosphorylation	Pyrophosphorylation
Electron donor	Electron acceptor	Proton	Neutron
Nitrogen	Carbon	Hydrogen	Phosphate
Force	Action	Metabolism	Energy
Break up	Break down	formation	reduction
Pyruvic acid	Lactic acid	Nucleic acid	Acidic acid
O ₂	N ₂	H ₂	CO ₂
	4	2	3
			1
Respiration	Fermentation	Oxidation	Carboxylation
TCP cycle	EMP pathway	Fermentation	Pentose phosphate shunt
Methionine	Cysteine	Cystine	Valine
Dissimilation of nutrients	Energy is not required	Synthesis of cell components	Dissimilation is an energy requiring process
An ion	A proton	A neutron	An electron
Flagella	ATP	Protein	Hydrogen atom
Photophosphorylation	Oxidative phosphorylation	Substrate level phosphorylation	Cyclic phosphorylation
Embden-Meyerhoff pathway	Pentose Phosphate pathway	Glycolytic pathway	Entner-Doudoroff pathway
More	Equal	Less	Abundant
Cyanobacteria	Marine bacteria	Photosynthetic bacteria	Iron bacteria
Oxidative phosphorylation	Kreb's cycle	EMP pathway	Substrate level phosphorylation
ED pathway	EMP pathway	Kreb's cycle	Fermentation
Glycolytic portion	Oxidative portion	fermentative portion	Kreb's portion
. Phosphates	CO ₂	Sugar	Nitrates
Active protein surface	Ammonium phosphate	Adenosine phosphate	Adenosine potassium sulfur

PAPS	ATP	ADP	AMP
UTP	CTP	AMP	PAPS
Phosphofructo kinase	Phosphohexo kinase	Lipase	Epimerase
ADP	ATP	AMP	FAD
Anabolic pathway	Catabolic pathway	Biological pathway	Glycolytic pathway
Acetoin	Acetyl CoA	Dihydroxyacetone phosphate	Flavoprotein
a. In e ⁻ transport chain	a. In e ⁻ transport chain	Enters EMP pathway	Enters HMP pathway
12,000	10,000	8,000	5,000
Plastocyanin	phytocyanin	Plastobillin	Phycobillin
galactose	pyruvate	oxaloacetate	malate
In e ⁻ transport chain	In e ⁻ transport chain	Enters EMP pathway	Enters HMP pathway
NADPH	NADP	NADPH ₂	NADH
Cytochrome	Auxochrome	Dichrome	Methanochrome
PRPP	RRP	PPR	RPRR
Nicotin amide nucleotide	Biotin amide nucleotide	Biotin by-products	Nucleotides
Ubiquinone.	Monoquinone	Cytochrome c	Cytochrome f
PMF	UTP	GTP	GDP
Z pathway	Glyoxalate pathway	Non-cyclic pathway	EMP pathway
Photosystem I	Photosystem II	Photosystem I & II	None
Z pathway	PMF	Photoreaction center	Non-cyclic pathway
Non-cyclic pathway	Z pathway	Both a and b	Photoreaction center
glycolysis	TCA cycle	ED pathway	HMP

ANSWER KEY

Pseudomonas

1

2

HMP pathway

transketolase

cytoplasmic matrix

Photophosphorylation

Hexokinase

Phosphofructokinase

Substrate level phosphorylation

6-phosphofructokinase

ATP

Ethanol & CO₂

Adenine

EMP pathway

Phosphorylation

Electron donor

Phosphate

Energy

Break down

Pyruvic acid

O₂

1

Respiration

Pentose phosphate shunt

Methionine

ring process Dissimilation of nutrients provides the building blocks for the synthesis of cell constituents

An electron

ATP

Substrate level phosphorylation

Glycolytic pathway

Less

Marine bacteria

Substrate level phosphorylation

EMP pathway

Glycolytic portion

sugar

Adenosine phosphosulfate

PAPS

UTP

Lipase

AMP

Catabolic pathway

Dihydroxyacetone phosphate

a. In e^- transport chain electron enters photosystem II
12,000

Plastocyanin

pyruvate

Enters EMP pathway

NADP

Methanococcus

6000

PRPP

Ubiquinone.

PMF

Non-cyclic pathway

Photosystem I

Photoreaction center

Z pathway

glycolysis

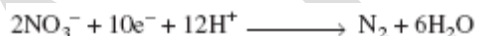
uents.

UNIT – 4**ANAEROBIC RESPIRATION AND FERMENTATION****Anaerobic Respiration**

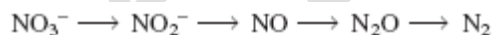
Electrons derived from sugars and other organic molecules are usually donated either to endogenous organic electron acceptors or to molecular O₂ by way of an electron transport chain. However, many bacteria have electron transport chains that can operate with exogenous electron acceptors other than O₂. This energy-yielding process is called anaerobic respiration. The major electron acceptors are nitrate, sulfate, and CO₂, but metals and a few organic molecules can also be reduced. Some bacteria can use nitrate as the electron acceptor at the end of their electron transport chain and still produce ATP. Often this process is called dissimilatory nitrate reduction. Nitrate may be reduced to nitrite by nitrate reductase, which replaces cytochrome oxidase.



However, reduction of nitrate to nitrite is not a particularly effective way of making ATP; because a large amount of nitrate is required for growth (a nitrate molecule will accept only two electrons). The nitrite formed is also quite toxic. Therefore nitrate often is further reduced all the way to nitrogen gas, a process known as denitrification. Each nitrate will then accept five electrons, and the product will be nontoxic.



There is considerable evidence that denitrification is a multistep process with four enzymes participating: nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase.



Interestingly, one of the intermediates is nitric oxide (NO). In mammals this molecule acts as a neurotransmitter, helps regulate blood pressure, and is used by macrophages to destroy bacteria and tumor cells. Two types of bacterial nitrite reductases catalyze the formation of NO in bacteria. One contains cytochromes *c* and *d1* (e.g., *Paracoccus* and *Pseudomonas aeruginosa*), and the other is a copper protein (e.g., *Alcaligenes*). Nitrite reductase seems to be periplasmic in gram-negative bacteria. Nitric oxide reductase catalyzes the formation of nitrous oxide from NO and is a membrane-bound cytochrome *bc* complex. Example of denitrification is gram-negative soil bacterium *Paracoccus denitrificans*, which reduces nitrate to N₂ anaerobically. Its chain contains membrane-bound nitrate reductase and nitric oxide reductase, whereas nitrite reductase and nitrous oxide reductase are periplasmic. The four enzymes use electrons from coenzyme Q and *c*-type cytochromes to reduce nitrate and generate PMF. Denitrification is carried out by some members of the genera *Pseudomonas*, *Paracoccus*, and *Bacillus*. They use this route as an alternative to normal aerobic respiration and may be considered facultative anaerobes. If O₂ is present, these bacteria use aerobic respiration (the synthesis of nitrate reductase is repressed by

O₂). Denitrification in anaerobic soil results in the loss of soil nitrogen and adversely affects soil fertility. Two other major groups of bacteria employing anaerobic respiration are obligate anaerobes. Those using CO₂ or carbonates as a terminal electron acceptor are called methanogens because they reduce CO₂ to methane. Sulfate also can act as the final acceptor in bacteria such as *Desulfovibrio*. It is reduced to sulfide (S₂ or H₂S), and eight electrons are accepted.



Fermentation

Fermentation is an alternative energy yielding process for respiration, which is preferred by organisms that are facultative or obligate anaerobes. Respiration is the most common energy yielding process in all organisms; the prerequisite being the presence of oxygen, and hence, referred to as aerobic cellular respiration. However, fermentation occurs totally in the absence of oxygen, and yields energy from oxidation of organic compounds (mainly sugars). This process is commonly carried out by yeast cells, or by some bacteria to produce certain types of dairy products like cheese and yogurt, and alcoholic beverages like wine, brandy, alcohol, rum etc. Fermentation is mainly of three types, and commonly, it is classified under 2 categories; alcoholic and lactic acid. The former occurs when the byproduct pyruvate is converted to ethanol and carbon dioxide. On the other hand, in the latter type, pyruvate is converted to lactic acid.

Alcohol fermentation

The Process of Alcohol Fermentation

The basic equation for alcohol fermentation shows that yeast starts with glucose, a type of sugar, and finishes with carbon dioxide and ethanol. However, to better understand the process, we need to take a look at some of the steps that take us from glucose to the final products.

The process of alcohol fermentation can be divided into two parts. In the first part, the yeast breaks down glucose to form 2 pyruvate molecules. This part is known as glycolysis. In the second part, the 2 pyruvate molecules are converted into 2 carbon dioxide molecules and 2 molecules of ethanol, otherwise known as alcohol. This second part is called fermentation.

The main purpose of alcohol fermentation is to produce **ATP**, the energy currency for cells, under anaerobic conditions. So from the yeast's perspective, the carbon dioxide and ethanol are waste products. That's the basic overview of alcohol fermentation. Now, let's examine each part of this process in greater detail.

In the first part of this process, each glucose molecule is broken down into 2 pyruvate molecules. Pyruvate, or pyruvic acid, is an amino acid and will help form ethanol. In the process of breaking glucose down to form pyruvate, several molecules known as electron acceptors are involved.

Electron acceptors are molecules whose job is to give and take the electrons released when a chemical reaction takes place. During this first part, an electron acceptor molecule called NAD⁺ is reduced to form NADH, gathering up the electrons released by breaking one glucose down to

2 pyruvate molecules. This exchange of electrons that occurs while glucose is being broken down is essentially what helps build ATP.

The conversion of glucose to pyruvate creates a net total of 2 ATP. While this isn't as much ATP as aerobic respiration can produce, it's enough to keep the yeast alive until oxygen is available. This first part may look familiar because it's essentially glycolysis, or the first stage of aerobic respiration.

If oxygen were present, then the pyruvate molecules would enter a mitochondrion to undergo the remainder of aerobic respiration. However, in alcohol fermentation, the pyruvate instead stays in the **cytosol**, the gooey interior space of the cell. This is where the second part of our reaction, the conversion of pyruvate to ethanol, will take place.

Before pyruvate can be converted to ethanol, it is first converted into an intermediary molecule called acetaldehyde. This releases carbon dioxide. Next, acetaldehyde is converted into ethanol. Key enzymes aid in the conversion of pyruvate to carbon dioxide and ethanol, including the zymases.

Lactate fermentation

Aerobic Respiration

Firstly, we have to understand the steps of aerobic respiration, since fermentation is a type of anaerobic respiration. In aerobic animals, respiration involves 2 pathways: glycolysis and citric acid cycle. These cycles involve the production of energy in the form of ATP (Adenosine Triphosphate) by breaking down the sugars (mainly glucose - as it is the simplest form of sugar). Glycolysis is a process involving a series of redox reactions to convert glucose into pyruvate or pyruvic acid; one of the products of glycolysis (end product). Pyruvic acid enters the Krebs cycle, and produces energy with the help of NADH molecules (co-factors that help to generate energy). Energy production actually occurs on the F1 particles situated on the cristae of mitochondria, wherein NADH is alternatively oxidized and reduced with the release of H⁺ ions/protons, which set up a gradient/flux to generate ATP. The resultant electrons are accepted by oxygen, and water is produced as a byproduct.

Steps of Lactic Acid Fermentation

Fermentation is a two step process, the first being anaerobic glycolysis, up till the formation of pyruvate. The pathways then change because of the available substrates and acceptors, and prevailing of specific environmental conditions. Fermentation of lactic acid is generally carried out by anaerobic bacteria and yeast. The following paragraphs explain this process along with the lactic acid fermentation formulas.

Homolactic Fermentation

In this type, glucose is converted to pyruvate, which further generates 2 lactic acid molecules with the aid of the enzyme lactate dehydrogenase.



Heterolactic Fermentation

This involves the use of pyruvate to produce lactic acid, ethanol, and carbon dioxide as byproducts, under the aid of the enzymes lactate dehydrogenase and pyruvate decarboxylase.



Fermentation of lactic acid has wide applications in the food and beverage industries.

- Production of this acid is commonly carried out by the lactic acid bacteria, *Lactobacillus spp.*, for production of cheese, yogurt, sauerkraut, bread, and kefir, and for imparting a peculiar sour taste to such food items.
- All beverage industries use the above described fermentation mechanism to produce wines, alcohol, beer, brandy, and other beverages.
- According to research, lactic acid products are high in vitamins and essential nutrients, contrary to their normal counterparts, and hence, are healthy to consume.

Concept of linear and branched pathways

Linear pathways convert one compound through a series of intermediates to another compound. An example would be glycolysis, where glucose is converted to pyruvate. Branched pathways may either be divergent (an intermediate can enter several linear pathways to different end products) or convergent (several precursors can give rise to a common intermediate). Biosynthesis of purines and of some amino acids are examples of divergent pathways. There is usually some regulation at the branch point. The conversion of various carbohydrates into the glycolytic pathway would be an example of convergent pathways.

Unit – IV
Possible Questions

Two Marks

1. Define fermentation.
2. Write short notes on aerobic fermentation.
3. Write short notes on dissimilatory nitrate reduction.
4. Distinguish between aerobic and anaerobic respiration in microbes.
5. Define de-nitrification.
6. Define uncouplers.
7. Define inhibitors.

Eight Marks

1. Explain nitrogen cycle.
2. Give an account on nitrogen fixation.
3. How is lactate fermentation carried out in microbes? Explain.
4. Explain in detail about the process involved in alcoholic fermentation.
5. Outline the steps involved in the ammonia respiration.
6. Give an account on Uncouplers and Inhibitors.

UNIT IV

Covertion of acid into aminoacid is called ____
Amine group comes from preexisting aminoacid is called ____
The joining of aminoacids to form proteins ____
peptidoglycan monomers are synthesised in ____
Peptidoglycan layer is ____ in gram positive than Gram negative bacteria
Peptidoglycan in the bacterial cell wall is ____
Thickness of gram positive cell wall ____
Archae bacteria have ____
Murein is a ____
The Phosphatidic acid intermediate of phospholipids synthesis is activated by ____
The process of nitrogen fixation requires energy from ____.
The amino acid ____ can be formed from the reaction of ammonium ions with ____
The amino acid L- glutamate can be formed from the reaction of ammonium ions wi ____
The ability to transform the amino group of one amino acid to form another amino a ____ serves as the nitrogen source for all the amino acids.
L-glutamate can react with 3- phosphoglycerate an intermediate of the glycolytic pat ____ is a precursdor for the biosynthesis of the amino acid L-glycine and L- cy ____ is a sulfur containing amino acid.
The transformation of L-serine to L- cystein involves a reaction with ____.
The formation of the aromatic ring structure involving the intermediate metabolite ____
During the biosynthesis of pyrimidines ____ is formed from aspartate and carb: ____ is a nucleotide in DNA and RNA.
Carbon dioxide and methyl group donated from ____ are also essential for the for ____
Biosynthesis of the adenine ring involves the substitution of an ____ group for ____
____ metabolic steps are involved in the formation of the basic purine ring struc ____
____ is the most important of the transaminase enzyme.
The biosynthesis of peptidoglycan is essential for cell growth and ____ of bacter ____
Peptidoglycan is a polysaccharide composed of N-acetylglucosamine and ____.
The enzyme that adds PEP to N-acetylglucosamine UDP is inhibited by ____ a ____
The assembly of precursdors of peptidoglycan during cell wall synthesis takes place ____
The pyrophosphate is specifically inhibited by the ____ antibiotic.
The translocation step of peptidoglycan synthesis is inhibited by the antibiotic ____
Several enzymes involved in peptidoglycan synthesis bind to penicillin are called ____
In lipopolysaccharide biosynthesis ____ serves as the lipid carrier.
The lipid A layer of lipopolysaccharide is assembled in the ____.
The fatty acid molecule seen in lipopolysaccharide is ____.
The transfer of LPS molecules to the outer layer is done through ____.
Extracellur proteins that aid in the establishment and maintainance of disease are cal ____

The enzyme that uses the phospholipid lecithin as the substrate is called _____.
_____ are the lytic agents capable of lysing white blood cells and may decrease
_____ serves as lipid carrier in the synthesis of peptidoglycon
_____ connects the cytoplasmic membrane and outer membrane in gram -ve ba
The biosynthesis of lipopolysaccharides occur at _____
The peptidoglycon layer of Gram negative bacteria is located in the _____
EMP pathway otherwise called as _____
_____ are components of nucleic acid
Pyrimidine is the components of _____
PRPP is a _____ donor
Purines are one of two families of nitrogen-containing molecules called _____
Gram positive Cell wall assembly is catalyzed by _____
Synthesis of lipoteichoic acid is occur on the surface of the _____
R5P normally derived from _____
Gram negative bacteria must transport _____
Attachment of the completed teichuronic acid to peptidoglycan apparently occurs by
_____ is generally accepted as the energy provider for transpor
_____ between murein strands, the gram positive wall is inherently

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OPTION A	OPTION B	OPTION C	OPTION D
amination	transcription	translation	transamination
amination	transcription	translation	transamination
require ATP	loss energy	loss ATP	require GTP
prenol	cytoplasma	cell	cytosol
larger	lesser	thicker	smaller
thin layer structure	tri crystal structure	cuboidal structure	crystal lattice structure
10-20nm	20-80nm	80-100nm	10-50nm
Pseudopeptidoglycan	murein	peptidoglycan	peptide
protein	polymer	amino acid	lipid
Cytosine triphosphate	Pyruvic acid	Flavoprotein	Malonyl CoA
ADP	AMP	ATP	FAD
D-glutamate	L-Lysine	L-glutamate	Mesodipimelic acid
Reductive amination	Phosphorylation	Fermentation	Fixation
Fermentation	Trans amination	Phosphorylation	Reductive amination
D-glutamate	L-glutamate	L-Lysine	Mesodipimelic acid
D-glutamate	L-Lysine	L-glutamate	L-serine
L-serine	Mesodipimelic acid	L-glutamate	L-Lysine
L-Lysine	L- cystein	L-serine	L-glutamate
Hydrogen sulfide	Ammonia	Sulphuric acid	Methane
Shikimic acid	Formic acid	Sulphuric acid	Acetic acid
Adenosine phosphosulfate	Uridine triphosphate	Ammonium phosphate	Cytidine triphosphate
Uridine triphosphate	Adenosine phosphosulfate	Cytidine triphosphate	Ammonium phosphate
Formic acid	Folic acid	Butyric acid	Cytidic acid
Acidic	Keto	amino	alpha
	10	3	5
Epimerase	Cytidine triphosphate	Glutamate transaminase	Adenosine phosphosulfate
Division	Reproduction	Mating	Survival
Murine layer	Mesodipimelic acid	L-glutamate	N-acetylmuramic acid
Bacitracin	Phosphonomycin	Streptomycin	Dapsone
cytoplasm	Ribosome	Nucleus	Chloroplast
Streptomycin	Phosphonomycin	Bacitracin	Vancomycin
Bacitracin	Vancomycin	Phosphonomycin	Streptomycin
Penicillinase	Precursor of penicillin	Bactoprenol	Penicillin-binding protein
Bactoprenol	UTP	PAPS	Adenosine phosphosulfate
Nucleus	cytoplasmic membrane	Chloroplast	Ribosome
Folic acid	Formic acid	Beta-hydroxymyristic acid	Phosphoric acid
Adhesion sites	Peptidoglycan	Exosporium	Protoplast
Viral proteins	Virulence factors	Alpha toxin	Fibrins

Fructolipases	Phospholipids	Lecithinases	Leukocidins
Leukocidins	Fructolipases	Lecithinases	Phospholipids
N acetyl glucose amine	Vactoprenol	Galactose	Ethanolamine
LPS molecule	Beyer junction	Biotin	Hydroxyl butarate
Nuclear membrane	Plasma membrane	Cytoplasmic membræ	Periplasmic space
Triplasmic	Metaplasmic	Periplasmic	Megaplasmic
glycolysis	TCA cycle	ED pathway	HMP
aminoacid	protein	carbon	purine
cell wall	nucleic acid	Cytoplasmic membræ	Mitochondria
Glucose	fructose	ribose	mannose
nitrogenous bases	protein bases	sugar bases	Nucleotides
Penicillin binding protei	protein	ferridoxin	Cofactors
membrane	cytoplasmic membræ	nucleus	cell membrane
Pentose phosphate pathv	EMP pathway	ED pathway	TCA cycle
murein	Lipopolysaccharide	murein precursors	pseudomurein
phosphodiester linkage	diester bond	ester bond	phosphoric linkage
proton force	Proton motive force	transport chain	electron transport chain
peptidoglycan content	Lipopolysaccharide	murein precursors	Cofactors

ANSWER KEY

amination
transamination
require ATP
cytosol
thicker
crystal lattice structure
20-80nm
Pseudopeptidoglycan
polymer
Cytosine triphosphate
ATP
L-glutamate
Reductive amination
Trans amination
L-glutamate
L-serine
L-serine
L- cystein
Hydrogen sulfide
Shikimic acid
Uridine triphosphate
Cytidine triphosphate
Folic acid
amino
10
Glutamate transaminase
Division
N-acetylmuramic acid
Phosphonomycin
cytoplasm
Bacitracin
Vancomycin
Penicillin-binding protein
Bactoprenol
cytoplasmic membrane
Beta-hydroxymyristic acid
Adhesion sites
Virulence factors

Lecithinases
Leukocidins
Vactoprenol
Beyer junction
Cytoplasmic membrane
Periplasmic
glycolysis
purine
nucleic acid
ribose
nitrogenous bases
Penicillin binding protein
cytoplasmic membrane
Pentose phosphate pathway
Lipopolysaccharide
phosphodiester linkage
Proton motive force
peptidoglycan content

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1. Cyclic Photophosphorylation:

When the photosystem I antenna chlorophylls funnel light energy to the reaction centre chlorophyll P_{700} , the latter gets excited and, as a result, its reduction potential becomes very negative. The excited or high-energy electron of P_{700} is captured by special chlorophyll 'a' molecule (A) or an iron sulphur protein (FeS).

The electron is eventually transferred to ferredoxin. The later transfer's electron to a cyclic route through a series of electron carriers (cytochrome $b_{563} \rightarrow$ plastoquinone \rightarrow cytochrome $b_6 \rightarrow$ cytochrome $f \rightarrow$ plastocyanin) back to oxidized P_{700} .

Since the electrons travel in a cyclic pathway (i.e. they originate from P_{700} and come back to the P_{700}), the process is called cyclic photophosphorylation in which only photosystem I is involved. During cyclic phosphorylation, ATP is generated in the region of cytochrome b_6 .

2. Non cyclic photophosphorylation

In this photophosphorylation both photosystem I and II are involved. The reduction potential of P_{680} chlorophyll molecule of photosystem II is very electropositive, slightly more positive than that of the H_2O/O_2 couple. This facilitates the first step in oxygenic electron flow, the splitting of water (photolysis) into oxygen atoms ($1/82 O_2$) and hydrogen ions ($2H$). Photolysis donates an electron to the oxidized P_{680} molecule following the absorption of a quantum of light near 680 nm. The P_{680} molecule is now excited and reduces pheophytin 'a' which is chlorophyll 'a' without the magnesium atom. Electrons subsequently travel through quinone, plastoquinone, cytochrome b_6 (ATP is generated in the region of cytochrome b_6), cytochrome f and plastocyanin; the later donates electrons to photosystem I.

The electron is accepted by the oxidized reaction centre chlorophyll 'a' of photosystem I (P_{700}) which has previously absorbed light quanta and begin the steps to lead the reduction of NADP into NADPH.

Anoxygenic photosynthesis in Bacteria

Purple and green bacteria possess only photosystem I. Since they lack photosystem II, they cannot use water (H_2O) as an electron donor in noncyclic photophosphorylation (i.e., noncyclic electron transport) and thus cannot produce oxygen from water photosynthetically, i.e., they are anoxygenic.

Light Reaction in Purple Bacteria

Light-harvesting antenna bacteriochlorophyll molecules absorb light and transfer it to reaction centre bacteriochlorophyll called P_{870} (Fig. 25.5). P_{870} is excited and releases electron which proceeds to reduce a molecule of bacteriopheophytin (Bph) in the reaction centre. This transition completes very fastly taking about three-trillionth of a second (i.e., 3×10^{-12} sec.) time. Once

reduced, the bacteriopheophytin reduces several intermediate quinone (Q) molecules to finally, a quinone in “quinone pool”.

This transition is also very fast completing within less than one-billionth of a second. Electrons are now transported from the quinone through a series of iron-sulphur proteins (FeS) and cytochromes (Cyt) back to the reaction centre (P_{870}).

It is the cytochrome bc_1 complex that interacts with the quinone pool during photosynthetic electron flow as a proton motive force (PMF) used to derive ATP synthesis. In addition to ATP, NADP or NADPH are also produced by purple bacteria using H_2S (also $S_2O_3^{2-}$, S^0 and even Fe^{2+}) as external electron donors. When H_2S is the electron donor, globules of sulphur (S^0) are stored inside the cells of purple bacteria.

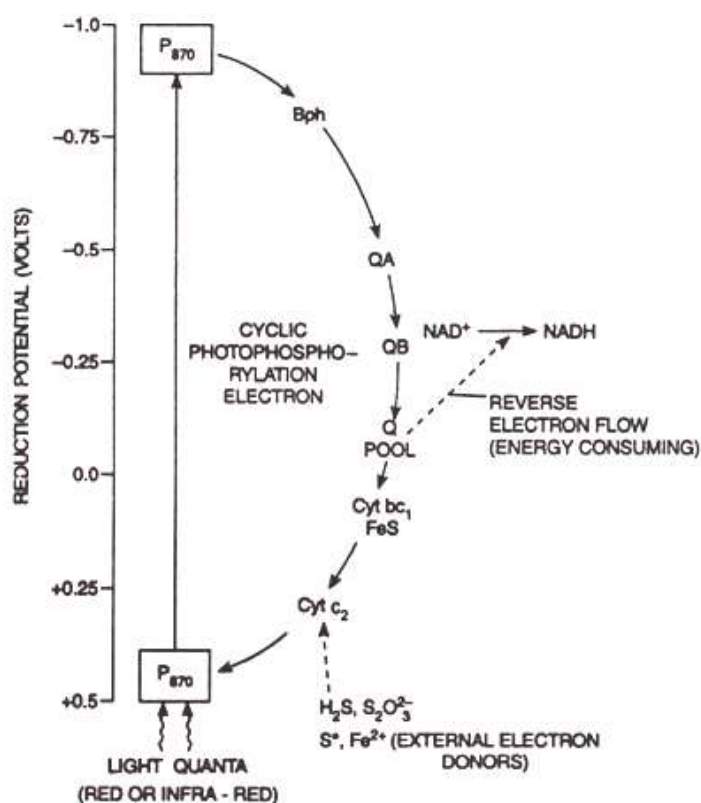


FIG. 25.5. Scheme of electron flow (cyclic photophosphorylation) in anoxygenic photosynthesis in purple bacteria.

A reversed electron flow operates in purple bacteria to reduce NAD^+ to NADH. The reduced H_2S or $H_2SO_3^{2-}$ (thiosulphate) are oxidized by cytochromes and electrons from them eventually end up in quinone pool. However, the energy potential of quinone is insufficiently negative to reduce NAD^+ directly. Therefore, the electrons from the quinone pool are forced backward to reduce NAD^+ to NADH. This energy requiring process is called reversed electron flow.

Light Reaction in Green Bacteria

The reaction centre bacteriochlorophyll is P_{840} that it absorbs light near 840 nm and resides at a significantly more negative reduction potential in comparison to purple bacteria.

Unlike purple bacteria where the first stable electron acceptor molecule resides at about 0.0 reduction potential, the electron acceptors of green bacteria (FeS proteins) reside at about -0.6 reduction potential and have a much more electronegative reduction potential than NADH.

In green bacteria, ferredoxin reduced by FeS protein serves directly as electron donor for dark reaction (fixation of CO_2). Thus, like oxygenic phototrophic microorganisms (and even green plants), in green bacteria both ATP and NADPH are direct products of light reactions.

When H_2S donates electrons to reduce NAD^+ to NADH in green bacteria, sulphur globules remain outside of the cell of green bacteria. This is unlike purple bacteria where the globules of sulphur remain inside of the bacterial cell.

Nitrogen metabolism

It is the polymeric nitrogen containing compounds proteins and nucleic acids that define the major attributes of organism such as function and structure. Operation and mechanism of metabolic pathways is provided by proteins.

Overview

It is the polymeric nitrogen containing compounds proteins and nucleic acids that define the major attributes of organism such as function and structure. Operation and mechanism of metabolic pathways is provided by proteins. Genetic information is stored in nucleic acid polymers. Each of the monomer of these macromolecules has an individual metabolic pathway. In addition, the monomeric nucleotides are essential for energy turnover as key intermediates in *all* metabolic pathways and also as second messenger molecules, often in form of cyclic nucleotides.

Amino acids contribute to carbohydrate synthesis via gluconeogenesis, to fat synthesis or energy Production via acetyl-CoA, and special nitrogen compounds such as catecholamines (neurotransmitters), thyroid hormones, creatine (-phosphate), the protoporphyrin ring (heme), and contribute to nucleic acid and phospholipid synthesis as nitrogen group donor.

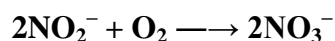
Nitrogen cycle

The nitrogen cycle involves three major steps: nitrogen fixation, nitrification, and denitrification. It is a cycle within the biosphere which involves the atmosphere, hydrosphere, and lithosphere. Instead, they depend on a process known as nitrogen fixation.

Process of nitrogen cycle

Ammonification: Ammonia is obtained from dead and decaying plants and animals by decomposition. This process is called ammonification.

Nitrification: In this step, ammonia obtained is first converted to nitrite (NO₂) by bacteria like Nitrosomonas, Nitrococcus, etc. and then to nitrate (NO₃) by Nitrobacterium. Bacteria involved in nitrification are called chemoautotrophs. Here is the reaction involved in the process of nitrification.



Denitrification

Once the nitrate is utilized by plants, the excess nitrate in the soil is reduced back to nitrogen by Pseudomonas and Thiobacillus bacteria. This process is known as denitrification.

Nitrogen Fixation

The concentration of usable form of nitrogen in the atmosphere is less. But certain bacteria called N₂-fixers help to fix this problem. Nitrogen fixation is the process in which diatomic nitrogen is converted into ammonia by bacteria like Rhizobium, Azotobacter, etc. The conversion is carried out by an enzyme called nitrogenase. Nitrogenase is an oxygen-sensitive enzyme which requires a strict anaerobic condition. A compound called leghaemoglobin acts as an oxygen scavenger and fulfills the demand of the enzyme.

The process of nitrogen fixation is initiated with the nodule formation. Rhizobium like bacteria divides and forms colonies around the root hairs and eventually invades them. There they produce nitrogen-fixing cells. The nitrogenase enzyme in the root nodule catalyzes the formation of ammonia. The whole process is carried out at the expense of ATP which is produced during plant [respiration](#).

Stages of nitrogen cycle

Microorganisms: Even though nitrogen has 78 percent share in the atmosphere, it is not in usable form for plants and animals. Here comes the role of microbes. Bacteria like, [Rhizobium](#) and blue-green algae convert this non-absorbable form of nitrogen to other compounds of nitrogen that are usable. These nitrogen compounds get fixed in soil by the microbes and the process is called nitrogen fixation. The natural phenomenon of lightning also helps in nitrogen fixation.

Plants: Plants absorb the usable nitrogen compounds from the soil. Their root system helps them in taking up nitrogen from the soil. Later, these nitrogen compounds are utilized for the synthesis of proteins and other nitrogen-containing compounds of cells.

Animals: We know that animals are dependent on plants for their food. While we feed on plants, these nitrogen compounds in plants get passed onto animals.

Unit – V
Possible Questions

Two Marks

1. Write about cyanobacteria.
2. Define photosynthesis.
3. What is methanogenesis? Give example.
4. What is meant by nitrogen fixation? Give example.
5. What is nitrogen cycle?
6. What is oxygenic photophosphorylation?
7. Define biological nitrogen fixation.

Eight Marks

1. Give an account on methanogenesis process.
2. Explain about biological nitrogen fixation.
3. Describe the process of anoxygenic photosynthesis?
4. Explain the nitrogen cycle.
5. Explain in detail about oxygenic photosynthesis.

UNIT V

Photopigments associated with purple and green bacteria are _____

Anoxygenic photosynthesis is carried out by _____

Photosynthetic apparatus present in Cyanobacteria are _____

_____ organism is involved in the production of dextran from sucrose.

Bioluminescence involves the oxidation of a Luciferin in the presence of _____

_____ is light produced by a chemical reaction in an organism.

_____ is the commonest cause of luminescence in the surface water of _____

_____ is a large protein which make up 2-5-% of soluble protein in _____

In photolithotrophic microbes ATP is used in synthesis of cell constituent from _____

BGA the source of H_2 is H_2O which is thereby oxidized to _____

An example of direct photoassimilation of an organic substrate is _____

In the dark, some of the photoorganotrophic bacteria oxidise organic substrate through _____

In photosystem I & II conversion of light energy in to _____ energy occurs.

The reactive pigment in photosystem I is _____

The pigment used in photosystem I is _____

The standard reduction potential of the reaction centre in photosystem is _____

Electrons expelled from photosystem I are accepted by _____ molecules.

Example for prokaryotic photosynthetic organism _____

Photosynthesis means _____

Example for green non sulfur bacteria _____

Example for green sulfur bacteria _____

Purple sulfur bacteria _____

Bacteriochlorophyll are located in _____

Other name for Thiobacteriaceae _____

Thiospirillum occurs in _____ shape

Non-motile form of Rhodospirillaceae is _____

Organic compound is utilized by _____

H_2S is utilized by _____

Example for oxygenic photosynthesis _____

Gas vacuoles are needed for _____

Green sulfur bacteria exist in _____ rich zone lakes

The Oxidation of ethanol was strictly _____ dependent

_____ using sulfate as terminal e acceptor

Enzyme catalyzing sulfate to adenosine 5 – phosphosulfate is _____

The process of conversion of light energy from the sun to chemical energy with in _____

The membrane bound carriers are collectively known as _____

The light is captured by light harvesting _____ pigments.
 An example for anoxygenic photoautotrophic bacteria is _____.
 _____ synthesis chlorophyll b in addition to chlorophyll a.
 The vesicles produced by green photoautotrophic bacteria is known as _____.
 _____ use organic acid as electron donors.
 When cyanobacteria utilize _____ they form elemental sulphur granules.
 Photosystem I is otherwise known as _____.
 How many protons are pumped during the passage of electrons through the carriers of p
 The protons used to reduce an oxidized carrier are known as _____.
 The reduced secondary quinone transfers its electrons to _____.
 Reaction center bacteria chlorophyll absorb maximally at _____.
 Phototrophic anoxygenic bacteria utilize _____ to generate NADH.
 Bacteria that utilize malate as electron donor is said to be _____.
 _____ aids in the motility of gliding bacteria.
 Sporulation takes place for 10 hrs in _____
 The synthesis of flagella involves _____ genes
 The information required for flagella construction is present in the structure of _____.
 Bacterial flagella anchor in to the cell wall and membrane by means of the -----
 ----- are membrane bound organelles in eukaryotic cells that contain very _____ 1
 Mycoplasma is an example of ----- bacteria
 Flagella of Spirochetes are called ----- flagella
 The peptidoglycan materials found in archae bacterial cell wall is called _____
 _____ is not a organic compound
 Phototrophy is the process by which organisms trap _____
 Cyanobacteria get their colour from the bluish pigment _____
 _____ highly visible blooms that can form in both freshwater and marine enviro

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OPTION A	OPTION B	OPTION C	OPTION D
Bacteriochlorophyll	Bacteriophytin	Cyanobacteria	Rhodophytin
Photosynthetic bacter	Cyanobacteria	Algae	Fungi
Chloroplasts	Thylakoids	Chlorophylls	Cytoplasm
<i>Bacillus subtilis</i>	<i>Pseudomonas aerogen</i>	<i>Streptococcus mutans</i>	<i>Proteus</i>
Luciferase	Protease	Cellulase	Ligase
Bioremediation	Biodeterioration	Bio degradation	Bioluminescence.
Dinoflagellates	Ctenophores	Cephalopods	Urochordates.
Cellulase	Ligase	Luciferase	Protease
Carbon monoxide	CO ₂	O ₂	N ₂
Molecular O ₂	CO ₂	O ₂	N ₂
<i>Rhodospirillum rubrum</i>	<i>Rhodospirillum rubrum</i>	Both	None
TCA cycle	Glycolysis	EMP pathway	Oxidation
Thermal	Chemical	Physical	Biological
Dual e ⁻ carrier	Single e ⁻ carrier	Protons carrier	Neutron carrier
P ₇₀₀	P ₅₀₀	P ₄₀₀	P ₆₀₀
450 mv	550 mv	650 mv	750mv
Ferredoxin	Ferredoxin	Ferredoxin	Ferrousdoxin
Cyanobacteria	Red algae	Higher plants	Lower plants
Light energy to chemi	Chemical energy to l	Light energy to physi	Physical energy to light energy
Chloroflexaceae	Chlorobiaceae	Chromatiaceae	Thiorodaceae
Chlorobiaceae	Chloroflexaceae	Thiorodaceae	Chlorobiaceae
Chlorobiaceae	Chromatiaceae	Thiorodaceae	Chloroflexaceae
Chlorosomes	Mesosomes	Metasomes	Ribosomes
Purple sulfur bacteria	Green sulfur bacteria	Green non sulfur bac	Green algae
Kidney	Heart	Liver	Round
Rhodocyclus	Rhodospirillum	Rhodococcus	Azospirillum
Green non sulfur bact	Purple sulfur bacteri	Green sulfur bacteria	Photosynthetic bacteria
Green sulfur bacteria	Purple sulfur bacteri	Green sulfur bacteria	Green non sulfur bacteria
Cyanobacteria	Blue green algae	Red algae	Green algae
Metabolism	Buoyancy	Catabolism	Transport
Sulfur	Iron	Copper	Nickel
H ₂	O ₂	N ₂	CO ₂
<i>Desulfovibrio</i>	<i>Methano bacterium</i>	<i>Nitrobacillus</i>	<i>Hydrogenomonas</i>
Pyrophosphatase	Sulfate adenylyl tran	Adenylyl sulfate kina	Sulfite reductase
Photophosphorylation	Substrate level phosph	Oxidative phosphory	Chemiosmosis
Oxidation reduction p	Photosystem	Chemiosmosis	Phosphorylation

Antenna	Flagella	Pili	Fimbriae
Cyanobacteria	Green bacteria	Purple sulphur bacteria	None
Cyanobacteria	Anabaena	Nostoc	Prochlorobacteria
Chlorosome	Vacuole	Centromere	Both a and b
<i>Pseudomonas sp.</i>	<i>Bacillus sp.</i>	<i>Rhodococcus sp.</i>	None
HCl	H ₂ S	H ₂ SO ₄	All the above
Z pathway	Non-cyclic oxidative	Cyclic oxidative photo	Photosystem I & II
8	12	16	4
Secondary quinine carrier	Primary quinine carrier	Tertiary quinine carrier	All the above
Cytochrome a	Cytochrome Bc ₁ complex	Cytochrome b	Both b and c
810nm	800nm	840nm	820nm
H ₂ S	H ₂ SO ₄	HNO ₃	NaCl
Photoorganotrophic	Photo autotrophic	Photo heterotrophic	Photolithotrophic
Slime	Fimbriae	Flagella	Pili
Streptococcus	Bacillus megaterium	Bacillus anthracis	Corynebacterium
20-30	40-50	15-30	30-40
Flagellin	hook	filament	basal body
Pilin	Stalk	Periplasm	Basal body
Mesosomes	Lysosomes	Metasomes	Trisomes
Gram negative	Cellwall high	Neutral	Cellwall less
Triplasmic	Periplasmic	Metaplasmic	megaplasmic
Glycopeptide	Mucopeptine	Pseudomurein	Muramic acid
fixed carbon	reduced carbon	organic carbon	aminoacid
chemicals	light energy	inorganic compounds	organic compounds
chlorophyll b	chlorophyll a	phycocyanin	xanthophyll
green sulphur bacteria	cyanobacteria	photosynthetic bacteria	fungi

ANSWER KEY

Rhophytin

Cyanobacteria

Thylakoids

. *Streptococcus mutans*

Luciferase

Bioluminescence.

Dinoflagellates

Luciferase

N₂

Molecular O₂

Rhodospirillum rubrum

TCA cycle

Chemical

Single e⁻ carrier

P₇₀₀

450 mV

Ferredoxin

Cyanobacteria

Light energy to chemical energy

Chromatiaceae

Chlorobiaceae

Thiorodaceae

Chlorosomes

Purple sulfur bacteria

Kidney

Rhodospirillum rubrum

Green sulfur bacteria

Purple sulfur bacteria

Green algae

Buoyancy

Iron

CO₂

Desulfovibrio

Pyrophosphatase

Photophosphorylation

Photosystem

Antenna

Purple sulphur bacteria

Cyanobacteria

Chlorosome

Rhodococcus sp.

H₂S

Cyclic oxidative phosphorylation

4

Secondary quinine carrier

Cytochrome Bc₁ complex

840nm

H₂S

Photoorganotrophic

Slime

Bacillus megaterium

20-30

Flagellin

Basal body

Lysosomes

Cellwall less

Metaplasmic

Pseudomurein

aminoacid

light energy

phycocyanin

cyanobacteria

12. _____ is the typical example of bacterium with rod shape.
 a) *Bacillus megaterium* b) *Streptococcus*
 c) *Corynebacterium* d) *Proteus*
13. Microbial population can be maintained in a state of exponential growth over a long period of time by _____
 a) Batch culture b) Continuous culture
 c) Synchronous culture d) Pure culture
14. The time required for the doubling of cell mass is known as _____
 a) Doubling time b) Generation time
 c) Generation gap d) Developing time
15. In _____ phase, rate of multiplication of bacteria increases with time.
 a) Lag b) Log
 c) Stationary d) Decline
16. The _____ of the microorganism is the time that it takes for the cell to reproduce.
 a) Growth curve b) Growth amount
 c) Growth rate d) Biomass
17. Reproduction of bacterial cells takes place by _____
 a) Pollination b) Binary fission
 c) Mitosis d) Meiosis
18. Growth rate is the reciprocal of _____
 a) Doubling time b) Cell division
 c) Binary fission d) Generation time
19. Microbial cultures composed of cells that are all the same stage of the cell cycle are called
 a) Axenic culture b) Pure culture
 c) Mixed culture d) Synchronous culture
20. In the atmosphere the availability of water is expressed as _____
 a) Relative humidity b) Xerotolerant
 c) Osmosis d) Water activity

Part - B Answer all the questions (3 X 2 = 6 Marks)

21. What is siderophore?
 22. Differentiate between lithotrophs and organotrophs.
 23. Define growth.

Part - C Answer all the questions (3 X 8 = 24 Marks)

24. a) Explain the nutritional types of microorganisms.
 (or)
 b) Describe passive diffusion and facilitated diffusion in terms of its distinctive characteristics and mechanisms.
25. a) Define growth and explain the different phases of microbial growth.
 (or)
 b) Explain the methods that are used for the measurement of microbial culture.
26. a) Explain the common nutrient requirements required for microbial growth.
 (or)
 b) Mention the processes involved in the uptake of nutrients by the cells and explain in detail about the active transport and group translocation.