KARPAGAM ACADEMY OF HIGHER EDUCATION

(Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

(For the candidates admitted from 2017 onwards)

DEPARTMENT OF MICROBIOLOGY

CLASS: I B.Sc (MB) **BATCH – 2019 -2022**

SUBJECT NAME: MICROBIAL PHYSIOLOGY AND METABOLISM

SUB.CODE:19MBU202 **SEMESTER: II** **4H – 4C**

SYLLABUS

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

COURSE 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.

COURSE OUTCOME (CO'S)

1. The students will be able to understand and predict the various metabolic reactions in microbial cell.
2. This will make them predict the intermediate products which can be employed in industrial production processes.

Unit I - Microbial nutrition

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 - Microbial growth

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

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

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 (homofermentative and heterofermentative pathways), concept of linear and branched fermentation pathways.

Unit V - Cyanobacteria

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. Talaro., Kathleen, P.T., Chess., and Berry, C., (2018). Foundations in Microbiology.(10th Ed).McGraw Hill Higher Education.
3. Moat, A.G., and Foster, J.W. (2002). Microbial Physiology. 4th edition. John Wiley & Sons.
4. Reddy, S.R., and Reddy, M. (2005). Microbial Physiology. Scientific Publishers India.
5. Gottschalk, G. (1986). Bacterial Metabolism. 2nd edition. Springer Verlag
6. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R. (2005). General Microbiology. 5th edition. McMillan.
7. Willey, J.M., Sherwood, L.M., and Woolverton, C.J. (2013). Prescott's Microbiology. 9thedition. McGraw Hill Higher Education.

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LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	Microbial nutrition - Nutrient Requirements	R1: 231-232
2	1	Nutritional group of microorganims	T8: 197-201
3	1	Uptake of nutrients by cell- Passive diffusion	T8: 197-201
4	1	facilitated diffusion	
5	1	Active transport	
6	1	Group translocation	
7	1	Iron uptake	
8	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit 1=08 hours		
		UNIT-II	
1	1	Different phases of growth curve and	T9:72-73
2	1	Generation time	T9:73-74
3	2	Measurement of microbial growth Batch	T9:68-74
4	3	Synchronous culture, Diauxic growth and Continuous culture	T9:69-75
5	3	Influence of environmental factor on growth Temperature, pH, solute, water activity, oxygen and pressure	R1:141-149
6	1	Recapitulation and discussion of question	

	Total No of Hours Planned For Unit II=11 hours		
		UNIT-III	
1	1	Carbohydrate metabolism - ED pathway	T9: 326-328
2	1	Carbohydrate metabolism – EMP pathway	T9: 330-331
3	1	Carbohydrate metabolism – Pentose phosphate pathway	T9: 329-330
4	1	Carbohydrate metabolism - TCA cycle	T9: 331-333
5	1	Aerobic respiration	R1: 243-244
6	1	Oxidative phosphorylation	R1: 235-237
7	2	Electron transport chain (Prokaryotic and Eukaryotic)	R1: 216-219
8	1	Substrate level phosphorylation	
9	1	Uncouplers and inhibitors	R2:440-460
10	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit III=11		
1	1	Anaerobic respiration - dissimilatory nitrate reduction	R1:247-248
2	1	Denitrification; nitrate/nitrite and nitrate	R1:275-278
3	1	Fermentative nitrate reduction	R1:247-248
4	2	Fermentation - Alcohol fermentation and Pasteur effect	R1:248-251
5	2	Lactate fermentation (homofermentative and heterofermentative pathways)	
6	1	Concept of linear and branched fermentation pathways	R1:248-251
7	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit IV=09		
1	2	Photosynthesis – bacteria and cyanobacteria	R1:257-262

2	1	photosynthetic pigments	
3	1	oxygenic (cyanobacterial) photosynthesis	
4	2	Anoxygenic (Purple, green bacteria) photosynthesis	T9:351-352
5	1	Nitrogen metabolism	R1:260-262
6	1	Recapitulation and discussion of question	
7	3	Old question paper discussion (Last Five years)	
	Total No of Hours Planned for unit V=11		

SUGGESTED READINGS:

T8: Michael J. Pelczar Jr., Chan E.C.S., Noel. R. Krieg. Microbiology (2004), Tata McGraw Hill Publishing Company Ltd, New Delhi, India.

T9: Dubey R C and Maheshwari D K. 2015. Textbook of Microbiology, S.Chand & Company Pvt Lmt.

R1: Prescott, Harley and Klein's. Microbiology, (2008), McGraw Hill International, 7th edition, New York, USA.

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

Nutritional Requirements of Microorganisms

Mineral Nutrients:

The microbial nutrients can be classified as macro (major) nutrients, and micro (minor) nutrients or trace elements on the basis of their amount required.

1. Macro or Major Mineral Nutrients:

The microbial cells contain water accounting for some 80-90% of their total weight and, therefore, the water is always the major essential nutrient in quantitative terms. The solid matter of cells contain, in addition to oxygen and hydrogen (derivable metabolically from water), the other macro (major) elements, namely, carbon, nitrogen, phosphorus, sulphur, potassium, magnesium, sodium, calcium and iron in order of decreasing abundance.

About 95% of cellular dry weight of microbial cells is accounted for only six macro (major) elements (O, H, C, N, P and S). However, approximate percentage of dry weight and general physiological functions of major mineral nutrients are given in Table.

Element (Nutrient)	Percentage of dry weight	Physiological functions
(*i*) Carbon (C)	50	Constituent of all organic cell materials.
(*ii*) Oxygen (O)	20	Constituent of cellular water and most organic cell materials; molecular oxygen serves as an electron receptor in aerobic respiration.
(*iii*) Nitrogen (N)	14	Constituent of proteins, nucleic acids, coenzymes.
(*iv*) Hydrogen (H)	8	Constituent of cellular water, organic cell materials.
(*v*) Phosphorus (P)	3	Constituent of nucleic acids, phospholipids, coenzymes.
(*vi*) Sulphur (S)	1	Constituent of some amino acids (cysteine and methionine), of some coenzymes (*e.g.*, CoA, cocarboxylase).
(*vii*) Potassium (K)	1	Important inorganic cation in cells, cofactor for some enzymatic reactions.
(*viii*) Sodium (Na)	1	Important inorganic cations in cells, important in membrane transport.
(*ix*) Calcium (Ca)	0.5	Important inorganic cation in cells, cofactor for some enzymatic reactions (*e.g.*, reactions by proteinases). It is essential component of endospores as calcium dipicolinate. Calcium concentrations affect membrane permeability and play a critical role in movement of flagella and cilia.
(*x*) Magnesium (Mg)	0.5	Important inorganic cation in cells, cofactor for some enzymatic reactions sometimes replacing Mg. Magnesium plays important role in protein synthesis; without it the ribosomal subunits do not associate and translation of nucleic acids into protein is not possible.
(*xi*) Iron (Fe)	0.2	Constituent of cytochromes and other haeme or non-haeme proteins, cofactor for a number of enzymatic reactions.

Approximate percentage of dry weight and general physiological function of mineral nutrients

Carbon assumes great importance as the main constituent of all organic cell materials and represents about 50% of cell's dry weight. CO2 is the most oxidized form of carbon and the photo-synthetic microorganisms reduce CO2 to organic cell constituents. On the other hand, all the non-photosynthetic microorganisms obtain their carbon requirement mainly from organic nutrients which contain reduced carbon compounds.

These organic compounds not only provide the carbon for synthesis but also meet the energy requirement by entering into energy yielding metabolic pathways and are eventually oxidised to CO2. Some microbes have the ability to synthesize all their cellular components using a single organic carbon source while others, in addition to this one major carbon source, also need other complex carbon containing components which they cannot synthesize.

These components are called growth factors and include vitamins. Some microbes can utilize more than one carbon compound and exhibit a great degree of versatility. The others, however, are specialized in this regard.

Sulphur and nitrogen are taken up by most organisms and are subsequently reduced within the cell and utilized in other biosynthetic processes. The sulphur and nitrogen requirements of most organisms can also be met with organic nutrients that contain these two elements in reduced organic combinations such as amino acids. A few microorganisms are capable of reducing elemental nitrogen to ammonia and this process of nitrogen assimilation is known as biological nitrogen fixation. Most of the microorganisms need molecular oxygen for respiration. In these, the oxygen serves as terminal electron acceptor, and such organisms are referred to as 'obligate aerobes'.

As opposed to this there are a few organisms which do not use molecular oxygen as terminal electron acceptor. We recall that oxygen is a component of the cellular material of all the microorganisms. These microbes are called 'obligate anaerobes'. In fact, molecular oxygen is toxic to these organisms. Aerobes which can grow in the absence of oxygen are called 'facultative anaerobes' and the anaerobes which can grow in the presence of oxygen are referred to as 'facultative aerobes'. In addition to these major classes, there are organisms which grow best at reduced oxygen pressure but are obligate aerobes and these are called 'Microaerophilic'.

2. Micro or Minor Mineral Nutrients or Trace Elements:

The microorganisms, in general do not use only macro (major) elements but also others like cobalt, copper, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc which are required in residual fraction by nearly all microorganisms. These elements are often referred to as minor (micro) nutrients or trace elements. The micronutrients or trace elements are nevertheless just as critical to cell function as are the macronutrients.

They are metals playing the role of cell's catalysts and many of them are play a structural role in various enzymes. Table summarizes the major micronutrients of living systems and gives examples of enzymes in

which each plays a role. Some microorganisms, however, need additional specific mineral nutrients, for example, diatoms and some microalgae require silica, supplied as silicate, to impregnate their cell walls.

Micronutrient	Cellular function
(*i*) Cobalt (Co)	Vitamin B_{12}; transcarboxylase (propionic acid bacteria).
(*ii*) Copper (Cu)	Respiration (cytochrome *c* oxidase); photosynthesis (plastocyanin, some superoxide dismutases).
(*iii*) Manganese (Mn)	Acts as activator of various enzymes; occurs in some superoxide dismutases and in the photolytic (water-splitting) enzyme in oxygenic phototrophs (photosystem-II).
(*iv*) Molybdenum (Mo)	Present in some flavin-containing enzymes, nitrogenase, nitrate reductase, sulphide oxidase, some formate dehydrogenases.
(*v*) Nickel (Ni)	Present in most hydrogenase enzymes; coenzyme F_{430} of methano-genes; carbon monoxide dehydrogenase; urease.
(*vi*) Selenium (Se)	Occurs in formate dehydrogenase; certain hydrogenases; amino acid selenocysteine.
(*vii*) Tungsten (W)	In some formate dehydrogenases; oxotransferases of hyperthermo-philes.
(*viii*) Vanadium (V)	Vanadium nitrogenase; bromoperoxidase.
(*ix*) Zinc (Zn)	In carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; many DNA-binding proteins.

Growth Factors:

Besides the mineral nutrients, the microorganisms need some organic compounds. Most of the microorganisms are capable of synthesizing these organic compounds from simpler carbon resources, others cannot and need their supply from outside for their proper growth and development.

Organic nutrients of this type are known collectively as growth factors (essential metabolites) and can be categorized into three groups (amino acids, purines and pyrimidines and vitamins) on the basis of their chemical structure and metabolic function.

Amino acids and purines and pyrimidines are the constituents of proteins and nucleic acids, respectively. Vitamins, however, are the most commonly needed growth factor and form parts of the prosthetic groups or active centres of certain enzymes. Some important vitamins and their functions are summarized in Table.

Since the growth factors fulfill specific needs in biosynthesis of certain molecules, they are needed in very small amounts; the vitamins even in less smaller quantities, because of the various coenzymes of which they are precursors, have catalytic roles and consequently are present at levels of a few parts per million in the microbial cell.

	Vitamin	Functions
(*i*)	Riboflavin (B_2)	Precursor of flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD) which are involved in electron transport chain.
(*ii*)	Cobalamine (B_{12})	Reduction of and transfer of single carbon fragments; synthesis of deoxyribose.
(*iii*)	Biotin	In fatty acid biosynthesis; in β-decarboxylation; in some CO_2-fixation reactions.
(*iv*)	*p*-Aminobenzoic acid	Precursor of folic acid.
(*v*)	Folic acid	One–carbon metabolism; transfer of methyl group.
(*vi*)	Thiamine (B_1)	Transketolase; α-decarboxylations.
(*vii*)	Nicotinic acid (niacin)	Precursor of nicotinamide adenine dinucleotide (NAD⁺); electron transfer in oxidation-reduction reactions.
(*viii*)	Lipoic acid	Acyl group transfer in decarboxylation of pyruvic acid and α-ketoglutaric acid.
(*ix*)	Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivations.
(*x*)	Vitamin B_6	Amino acid and keto acid transformations.
(*xi*)	Vitamin K	Electron transport; in synthesis of shingolipids.
(*xii*)	Hydroxamates	Solubilization of iron and transport into cell.

Nutritional Types of Microorganisms

Microbiologists use the term growth to indicate an increase in a population of microbes rather than an increase in size. Microbial growth depends on the metabolism of nutrients, and results in the formation of a discrete colony, an aggregation of cells arising from a single parent cell. A nutrient is any chemical required for growth of microbial populations. The most important of these are compounds containing carbon, oxygen, nitrogen, and/or hydrogen.All microorganisms require source of energy and hydrogen atoms or electrons for their growth and development. There are two sources of energy available to microorganisms, and based on this they are of two types:

Phototrophs – Energy for growth is derived from sunlight.

Chemotrophs – Energy for growth is derived from the oxidation of either organic and inorganic chemical compounds.

Microorganisms also have two sources for hydrogen atoms and electrons and based on that they can be grouped as:

Lithotrophs : Source of electrons is reduced inorganic compounds

Organotrophs : Source of electrons and hydrogen is organic compounds.

Although microorganisms show great metabolic diversity, yet most of them can be categorized in one of the four nutritional types based on energy, electrons or hydrogen and carbon sources. These types are :

1. **Photolithotrophic autotrophs**

Also called photoautotrophs as they use light as their source of energy and CO2 as their source of carbon. Some examples of this nutritional group are the eukaryotic algae and cynobacteria, purple sulphur bacteria and green sulphur bacteria.Among these, eukaryotic algae and cynobacteria use water as the electron donor

and release oxygen, whereas purple and green sulphur bacteria cannot oxidise water and thus, use the electrons from inorganic donors like hydrogen and hydrogen sulphide.

2. **Chemo-organotrophic heterotrophs**

These are also called as chemoheterotrophs. They use organic compounds like sugars and amino acids as source of energy,hydrogen and carbon. The vast majority of pathogenic microorganisms are chemoheterotrophs.

3. **Photo – organotrophic heterotrophs**

Some phototrophic bacteria like purple non sulphur and green non sulphur bacteria use organic compounds as electron donors and carbon sources. For example Rhodospirillum rubrum use succinate as an electron donor.

4. **Chemolithotrophic autotrophs**

They oxidise inorganic compounds like iron, sulphur or nitrogen to derive both energy and electrons for biosynthesis. Carbon dioxide is the carbon source for them. Few chemolihotrophs have been recognized to derive their carbon from organic sources and are called chemolithotrophic heterotrophs. Bacteria that obtain energy by utilizing inorganic electron donors but obtain most of their carbon from organic compounds are called mixotrophs.

Uptake of Nutrients

In order to support its' activities, a cell must bring in nutrients from the external environment across the cell membrane. In bacteria and archaea, several different transport mechanisms exist.

Passive Diffusion

Passive or **simple diffusion** allows for the passage across the cell membrane of simple molecules and gases, such as CO2, O2, and H2O. In this case, a concentration gradient must exist, where there is higher concentration of the substance outside of the cell than there is inside the cell. As more of the substance is transported into the cell the concentration gradient decreases, slowing the rate of diffusion.

Facilitated Diffusion

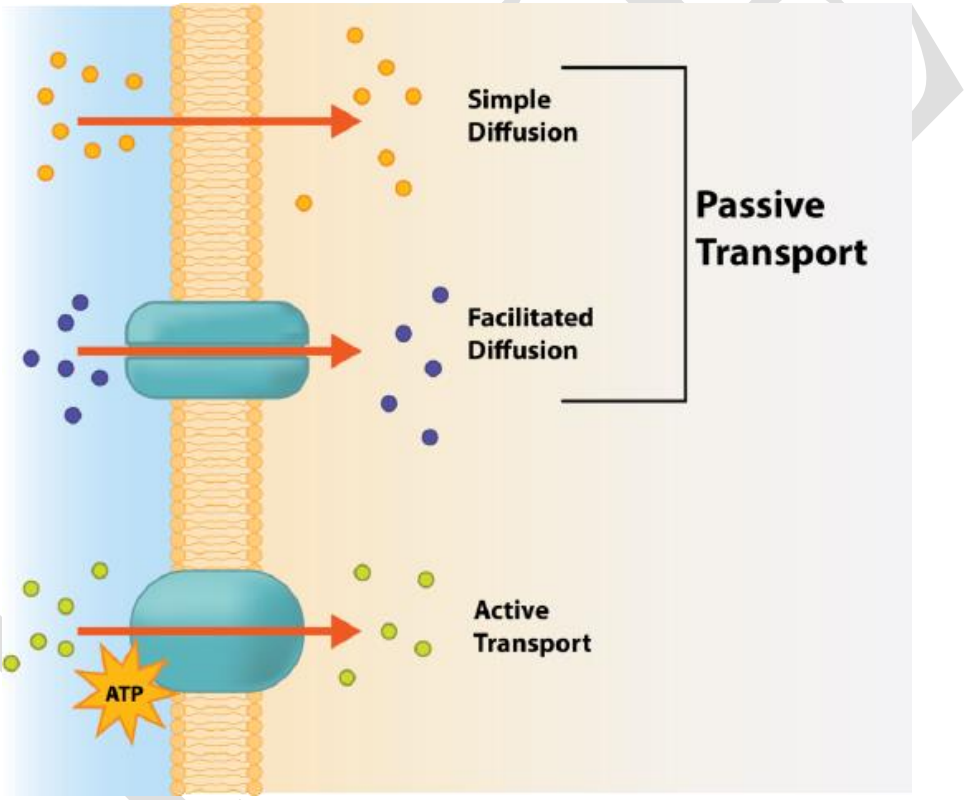
Facilitated diffusion also involves the use of a concentration gradient, where the concentration of the substance is higher outside the cell, but differs with the use of **carrier proteins** (sometimes called **permeases**). These proteins are embedded within the cell membrane and provide a channel or pore across the membrane barrier, allowing for the passage of larger molecules. If the concentration gradient

dissipates, the passage of molecules into the cell stops. Each carrier protein typically exhibits specificity, only transporting in a particular type of molecule or closely related molecules.

Active Transport

Many types of nutrient uptake require that a cell be able to transport substances against a concentration gradient (i.e. with a higher concentration inside the cell than outside). In order to do this, a cell must utilize metabolic energy for the transport of the substance through carrier proteins embedded in the membrane. This is known as **active transport**. All types of active transport utilize carrier proteins.

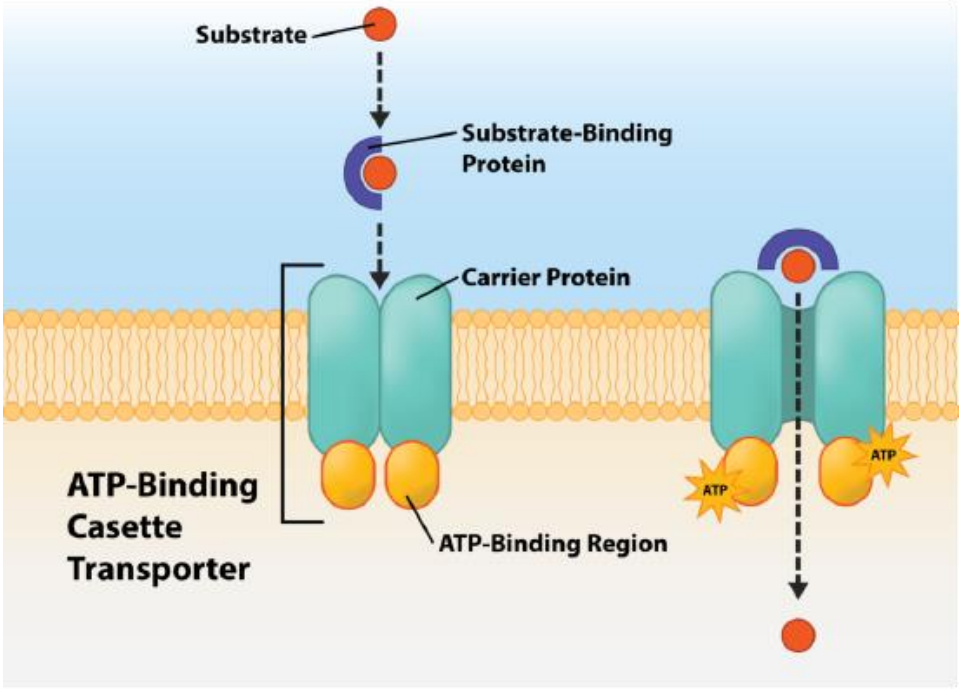
These terms can all be combined, to derive a single term that gives you an idea of what an organism is using to meet its basic needs for energy, electrons, and carbon.



Primary active transport

Primary active transport involves the use of chemical energy, such as ATP, to drive the transport. One example is the **ABC system**, which utilizes **ATP-Binding Cassette transporters**. Each **ABC transporter** is composed of three different components: 1) membrane-spanning proteins that form a pore across the cell membrane (i.e. carrier protein), 2) an ATP binding region that hydrolyzes ATP, providing the energy for the passage across the membrane, and 3) a substrate-binding protein, a peripheral protein that binds to the appropriate substance to be transporter and ferries it to the membrane-spanning proteins.

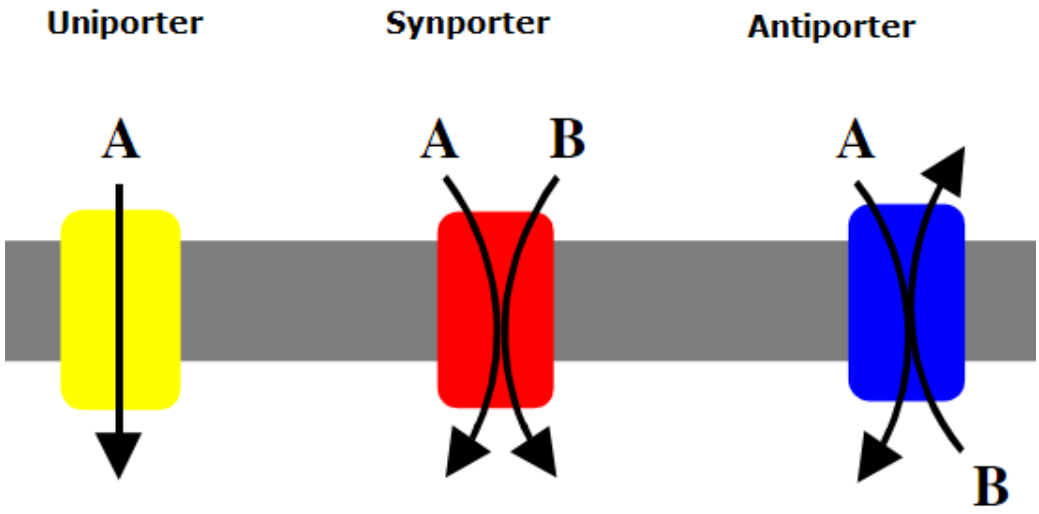
In gram negative bacteria the substrate-binding protein is located in the cell's periplasm, while in gram positive bacteria the substrate-binding protein is attached to the outside of the cell membrane.



Secondary active transport

Secondary active transport utilizes energy from a **proton motive force (PMF)**. A PMF is an ion gradient that develops when the cell transports electrons during energy-conserving processes. Positively charged protons accumulate along the outside of the negatively charged cell, creating a proton gradient between the outside of the cell and the inside.

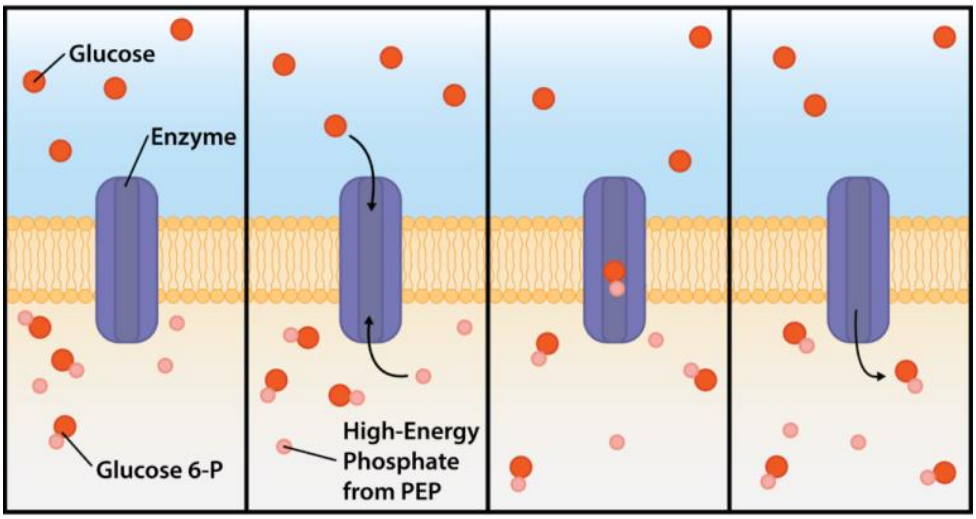
There are three different types of transport events for simple transport: **uniport**, **symport**, and **antiport** and each mechanism utilize a different protein **porter**. **Uniporters** transport a single substance across the membrane, either in or out. **Symporters** transport two substances across the membrane at the same time, typically a proton paired with another molecule. **Antiporters** transport two substances across the membrane as well, but in opposite directions. As one substance enters the cell, the other substance is transported out.



Group Translocation

Group translocation is a distinct type of active transport, using energy from an energy-rich organic compound that is not ATP. Group translocation also differs from both simple transport and ABC transporters in that the substance being transported is chemically modified in the process.

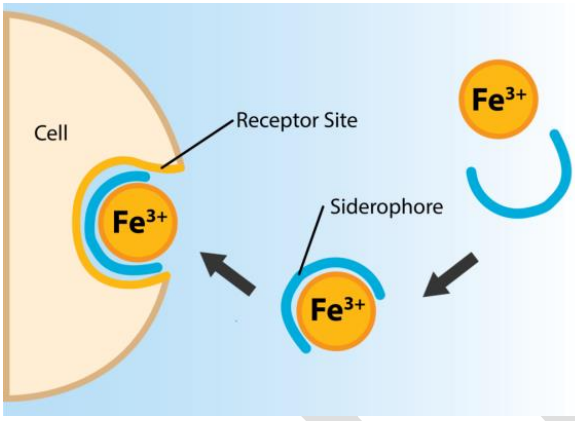
One of the best studied examples of group translocation is the **phosphoenolpyruvate: sugar phosphotransferase system (PTS)**, which uses energy from the high-energy molecule **phosphoenolpyruvate (PEP)** to transport sugars into the cell. A phosphate is transferred from the PEP to the incoming sugar during the process of transportation.



Group Translocation via PTS.

Iron Uptake

Iron is required by microbes for the function of their cytochromes and enzymes, resulting in it being a growth-limiting micronutrient. However, little free iron is available in environments, due to its insolubility. Many bacteria have evolved **siderophores**, organic molecules that chelate or bind ferric iron with high affinity. Siderophores are released by the organism to the surrounding environment, whereby they bind any available ferric iron. The iron-siderophore complex is then bound by a specific receptor on the outside of the cell, allowing the iron to be transported into the cell.



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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CLASS: I B.Sc., MB **COURSE NAME: MICROBIAL PHYSIOLOGY AND METABOLISM**

COURSE CODE: 19MBU202 **UNIT-I** **BATCH: 2019-2022**

S.no	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Vitamin B6 is otherwise called as .	riboflavin	Cyanocobalimine		Ribitol	pyridoxine
2	The end products of the mixed acid fermentation can be detected by the _____test.	MR test	VP test	Fermentation test	Nitrate reduction test	MR test
3	Photoautotrophic metabolism using light as the energy source and _____as a source of carbon	Glucose	Co2	Sucrose	Lactose	Co2
4	Permease involved in	diffusion	Active transport	pasive diffusion	facilitated diffusion	Active transport
5	Molecules are modified in	group translocation	Active transport		facilitated diffusion	group translocation
6	Ferric ion are	soluble	insoluble	immersible	disolved in water and solvent	insoluble
7	Microorganism use _____to uptake nutrients.	ferridoxin	chlorophyll	siderophores	light	siderophores
8	Peptone are	carbohydrate	lipid hydrolysate	protein hydrolysate	lipid	protein hydrolysate
9	Agar is a _____	Protein and DNA	lipid	Fatty acid	polysaccharide	polysaccharide
10	Constituent of cysteine	hydrogen	sulfur	oxygen	carbon	sulfur
11	Main constituent of cellular materials	hydrogen	sulfur	oxygen	carbon	carbon
12	Constituent of nucleic acid	hydrogen	phosphorous	oxygen	carbon	phosphorous

13	Cyanobacteria	chemoautotrophs	lithotrophs	photoautotrophs	chemotrophs	photoautotrophs
14	_____oxidize the inorganic compounds.	lithotrophs	lithotrophs	photoautotrophs	chemotrophs	lithotrophs
15	Carbon source for autotrophs	organic compounds	inorganic compounds	C	CO2	CO2
16	_____use organic form of carbon.	chemoautotrophs	lithotrophs	photoautotrophs	heterotrophs	heterotrophs
17	_____is the inorganic cellular cations.	hydrogen	oxygen	nitrogen	calcium	calcium
18	Which of the following defines a heterotrophs	obtain its carbon in an organic form	not require essential nutrients for growth	produce all trace elements	produce macroelements	obtain its carbon in an organic form
19	Molecules that satisfy heterotrophic nutritional requirements include all but which of the following?	lipid	Protein	water	carbohydrate	water
20	Primary source of nitrogen for heterotrophs include all except which of the following?	RNA	glucose	DNA	aminoacid	glucose
21	Needed in large amount of _____for cell metabolism	macronutrients	trace elements	micronutrients	nutrients	macronutrients
22	Passive diffusion _____	substance move from higher to lower	substance move from lower concentration to	substance move from outside to	substance move from inside to outside	substance move from higher to

		concentration	higher concentration	inside		lower concentration
23	Nitrogen is required for the production of what category of molecules?	Fattyacid	phospholipids	Nucleotide	Carbohyrates	Nucleotide
24	Shape of bacterial growth curve is ___	Straight	Curved	Sigmoid	Round	Curved
25	Bacterial species which grows as phototroph under anaerobic condition and as chemotroph under aerobic condition is _____	*Rhodospirillum rubrum*	*Rhodospirillum stratum*	Proteus	Vibrio	Proteus
26	Example of bacterial species which can grow either as chemolithotroph or chemoorganotroph is	*Pseudomonas pseudoflava*	*Pseudomonas putida*	*Pseudomonas fluroscence*	*Pseudomonas aeruginosa*	*Pseudomonas pseudoflava*
27	Chemolitho heterotrophs are also called as	Mixotroph	Auxotroph	Chemotroph	Lithotroph	Mixotroph
28	_____are organism that make use of carbondioxide as their main source of carbon.	Autotrophy	Heteotroph	Chemotroph	Lithotroph	Autotrophy
29	A solution in which water flows equally in to and out of a cell is termed _____solution	Isotonic	Monomeric	hypertonic	hypotonic	Isotonic
30	Members of archaeobacteria that	Red extreme halophiles	Blue extreme	Green extreme halophiles	Yellow extreme halophiles	Red extreme halophiles

	requires high salinity are called as _____		halophiles			
31	Shifts in pH in laboratory media can be prevented by incorporating a _____in to the medium	Acidic solution	Normal saline	Physiological saline	Buffer	Buffer
32	A solution in which water flows equally in to and out of a cell is termed _____solution	Isotonic	Monomeric	acidic	Neutral	Isotonic
33	The peptidoglycon layer of Gram negative bacteria is located in the ---------- space	Triplasmic	Metaplasmic	Periplasmic	Epiplasmic	Periplasmic
34	is the typical example of bacterium with rod shape.	Bacillus megaterium	Streptococcus	Corynebacterium	Proteus	Bacillus megaterium
35	Organisms that can not utilise oxygen gas are called as _____	obligate Aerobic organism	obligate Anaerobic organism	Facultatives aerobes	facultative anaerobes	oblicate Anaerobic organism
36	Water is important in the nutrition of microorganisms because the food of most microorganisms is _____in water.	Dissolved	Insoluble	Immersed	Diluted	Dissolved
37	Nitrogen is an essential element of	Amines	Aminoacids	Hydroxyl	Carboxyl	Aminoacids

	the _____that make up protein					
38	Cyanobacteria resemble green plants in that they evolve___as an end point of their metabolism.	N2	O2	CO2	H2	N2
39	Lipid content is more in the cell wall of Gram _____bacteria	Negative	Positve	Both	None	Negative
40	Volutin granules are also called as _____	Gas vacuoles	Mitochondria	Endoplasmic reticulum	Metachromatic granules	Metachromatic granules
41	Membrane invaginations in to the bacterial cytoplasm are known as	Mesosomes	Cytosomes	Hydroxysomes	Carboxysomes	Mesosomes
42	Microorganisms pathogenic for humans and other warm blooded animals grow best at a temperature of _____	40°C	37°C	35°C	20°C	37°C
42	In prokaryotic cells the region where DNA is located is referred to as_____	Nucleoid	Nuclear region	Nuclear body	Nucleosome	Nuclear region
43	Semi rigid extension of bacterial cell wall and cell membrane is called_____	Capsule	Stalk	Slime	Prosthecae	Prosthecae
44	Gas vesicles are mostly present in _____	Gram positive bacteria	Gram negative bacteria	Photosynthetic bacteria	Aquatic bacteria	Aquatic bacteria

45	Bacterial ribosomes are composed of	Protein and DNA	Protein and rRNA	Protein and mRNA	Protein and RNA	Protein and rRNA
46	The nuclear material in a bacterial cell is known as	Nucleus	Nucleoid	Nucleolus	Nucleosome	Nucleoid
47	When two molecules are entering the cell simultaneously in the same direction it is called……..	symport	antiport	translocation	active transport	symport
48	Phosphorus is essential element of the biosynthesis of _____as well as ATP.	Pyruvic acid	Lactic acid	Nucleic acid	Acidic acid	Nucleic acid
49	Which of the following mechanisms of transport doesn't involve substrate specific proteins?	Simple diffusion	Facilitated diffusion	Active transport	Primary active transport	Active transport
50	Water enters bacterial cell by	Facilitated diffusion	Passive diffusion	Active transport	Group translocation	Passive diffusion
51	Phototransferase system in bacteria is an example of	Facilitated diffusion	Active transport	Passive diffusion	Group translocation	Passive diffusion
52	_____use light as a source of energy and carbon dioxide as the main source of carbon	Chemoheterotrophs	Chemoautotrophs	Photoautotrophs	Photoheterotrophs	Photoautotrophs

53	95% of cell dry weight is made up of major elements such as C, O, H, N, S and P. These are called as	Macro elements	Micro elements	Accessory elements	elements	Macro elements
54	_____can use CO_2 as their sole source of carbon	Chemotrophs	Heterotrophs	Autotrophs	lithotrophs	Autotrophs
55	Microbes which grow only in the presence of free oxygen are called	Obligate aerobes	Obligate anaerobes	Facultative aerobes	Facultative anaerobes	Obligate aerobes
56	Diatoms and many algae are the examples of microorganism requiring vitamin	Biotin	Vitamin B12	Folic acid	Niacin	Vitamin B12
57	_____is the process in which molecules move from a region of higher concentration to lower concentration	Diffusion	Osmosis	Permease	All the above	Diffusion
58	Microorganism capable of growing at zero degree Celsius are called	Psychrophiles	Acidophiles	Alkalophiles	Mesophiles	Psychrophiles
59	_____are small organic molecules that usually make an all or part	Mineral	Vitamin	Fatty acid	proteins	Vitamin

	of enzyme cofactors					
60	Organisms that can not utilise oxygen gas are called as _____	obligate Aerobic organism	obligate Anaerobic organism	Facultatives aerobes	facultative anaerobes	obligate Anaerobic organism

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)

Bacterial growth curve

- The increase in cell number or growth in population is studied by analyzing the growth curve of a microbial culture. Bacteria can be grown or cultivated in a liquid medium in a closed system or also called as batch culture. In this method, no fresh medium is added and hence with time, nutrient concentration decreases and an increase in wastes is seen. As bacteria reproduce by binary fission, the growth can be plotted as the logarithm of the number of viable cells verses the time of incubation. The curve plotted shows four basic phases of growth; the lag, log, stationary, and death phase
- When fresh liquid medium is inoculated with a given number of bacteria and incubated for sufficient period of time, it gives a characteristic growth pattern of bacteria.
- If the bacterial population is measured periodically and log of number of viable bacteria is plotted in a graph against time, it gives a characteristic growth curve which is known as **growth curve or growth cycle.**
- The growth curve is hyperbolic due to exponential bacterial growth pattern.

Phases of Growth

During the lag phase, there is little or no change in the number of cells, but metabolic activity is high.

- DNA and enzyme synthesis occurs; may last from 1 hour to several days.

During the log phase, the bacteria multiply at the fastest rate possible under the conditions provided.

- Maintained by use of a chemostat – constant supply of fresh media

During the stationary phase, there is equilibrium between cell division and death.

- Nutrients are exhausted and waste products build up; pH increases.

During the death phase, the number of deaths exceeds the number of new cells formed.

The growth curve has following phases

1. Lag phase
2. Log phase or exponential phase
3. Stationary phase
4. Death phase or decline phase

1. Lag phase:

- When bacteria is inoculated into new fresh media, it do not divide immediately. Bacteria takes some time to adjust to the new environment. The time period in which bacteria is metabolically active but do not divide is called as lag phase.
- Lag phase is characterized by the period during which there is no increase in number of cell.

- Size of bacteria increase continuously so the bacteria have largest size at the end of lag phase.
- In this phase, microorganism tries to adopt in new environment. It is the phase of adjustment necessary for the synthesis of enzymes and co-enzymes for physiological activities.
- Time is required for adjustment in physical environment around each cell.
- Duration of lag phase varies according to conditions and species of bacteria.
- If the culture organism is taken from old culture, the duration will be longer but if the culture is fresh, duration is short.
- Similarly if the culture media is different from the previous culture then duration is long because bacteria takes some more time to adjust in the new media.
- At the end of lag phase, bacteria become fully prepared for cell division.

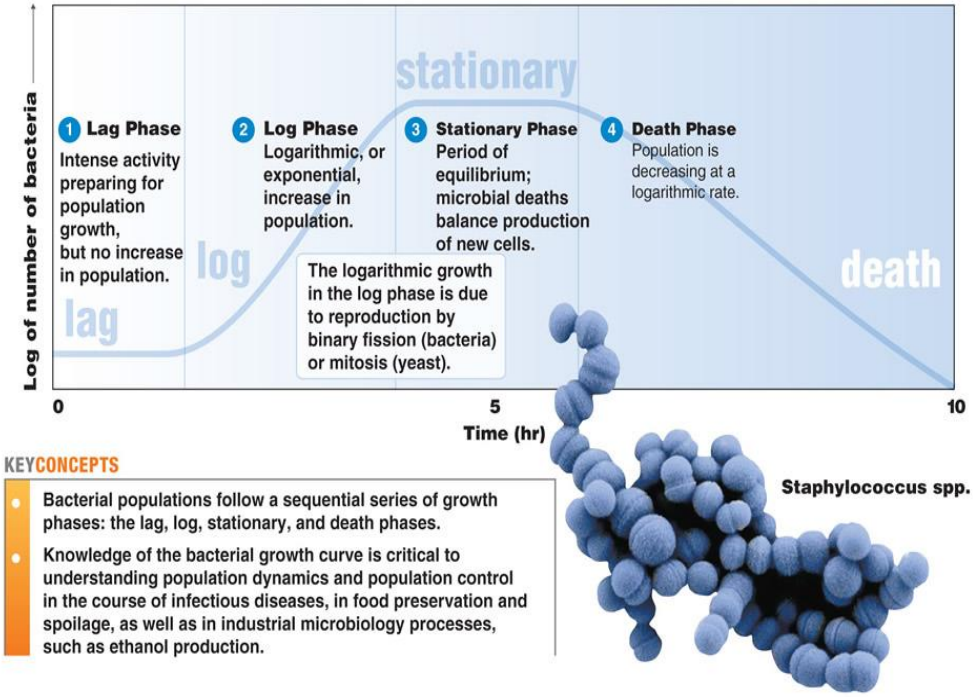


Figure: Bacterial growth curve

2. Log phase or exponential phase:

- During this phase bacteria divides continuously at constant rate and the number of bacteria increase exponentially.
- In this phase all bacteria are in their rapid stage of cell division and show balanced growth.
- Due to rapid cell division, bacteria have smallest size in this phase.
- Bacterial population is nearly uniform in terms of their metabolic activities, chemical composition of cell and other physiological characteristics.
- Biochemical and physiological characteristics are commonly used for identification of bacteria are manifested during log phase of growth.

- Generation time of bacteria is usually determined during log phase. However it is not same for all bacteria in culture.
- Generation time is shortest during log phase and is strongly dependent upon growth factors present in the medium.
- This phase lasts for several hour depending on the type of organism, conditions of growth and density of organism.

3. Stationary phase:

- The bacteria growth reaches a state during which there is no net increase in bacterial population. This is called as stationary phase.
- In this phase a constant bacterial population is maintained by balance between cell division and cell death.
- In some bacteria, complete cessation of cell division occurs hence there is no net increase or decrease in number of bacteria.
- Stationary phase is induced by- increased bacterial cell density, depletion of nutrition in media and accumulation of toxic secondary metabolic wastes.
- Production of antibiotics such as Penicillin, streptomycin etc and enzymes by certain bacteria occur during stationary phase of their growth.
- In endospore forming bacteria, sporulation occurs as the bacteria enter stationary phase.

4. Death phase or decline phase:

- In this phase, number of bacteria decrease continuously and exponentially.
- During this phase, total count of bacteria may remain constant but the viable count decreases.
- It is just inverse of log phase. But the death rate is slower than growth rate.
- Death phase is brought about by various reasons, such as depletion of nutrition and accumulation of toxic wastes.
- Not all bacteria die at same rate, some die faster and some are more resistant and remain viable for longer time. Eg. Spore forming bacteria.

Generation Time

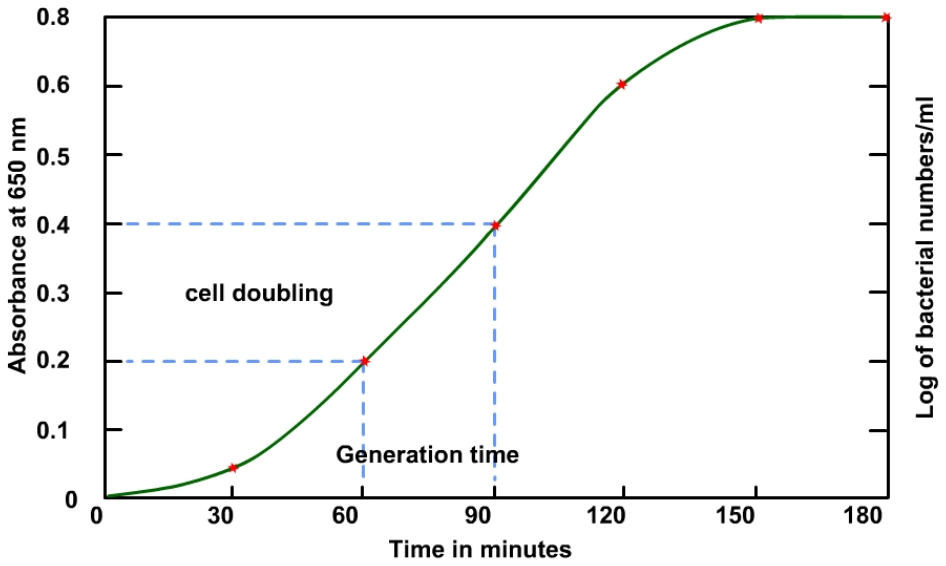
- The time required for a cell to divide or a population to double is known as the generation time.
- Most bacteria have a doubling time of 1-3 hours, although some may be greater than 24 hours.
- *E. coli* may have a doubling time of 20 minutes; get 20 generations in 7 hours, going from one cell to one million cells.

Table: Generation times for some common bacteria under optimal conditions of growth.

Bacterium	Medium	Generation Time (minutes)
Escherichia coli	Glucose-salts	17
Bacillus megaterium	Sucrose-salts	25
Streptococcus lactis	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast extract	344-461
Mycobacterium tuberculosis	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

CALCULATION:

The generation time can be calculated from the growth curve



Calculation of generation time

The exactly doubled points from the absorbance readings were taken and, the points were extrapolated to meet the respective time axis.

Generation Time = (Time in minutes to obtain the absorbance 0.4) – (Time in minutes to obtain the absorbance 0.2)

Generation Time = 90-60
= 30 minutes

Let No = the initial population number

Nt = population at time t

N = the number of generations in time t

Therefore,
$Nt = No \times 2^n$…… (1)

logNt = logNo + nlog2

Therefore,
n = (logNt - logNo) / log2

n = (logNt - logNo) / 0.301…… (2)

The growth rate can be expressed in terms of mean growth rate constant (k), the number of generations per unit time.

K = n/t

n = (logNt - logNo) / (0.301 X t)…… (3)

Mean generation time or mean doubling time (g), is the time taken to double its size.

Therefore,
Nt = 2No…… (4)

Substituting equation 4 in equation 3

k = (logNt - logNo) / (0.301 X t)

= (log2No - logNo) / (0.301 X t)

= log2 +(logNo - logNo) / (0.301 g)(Since the population doubles t= g)

Therefore,

K=1/g

Mean growth rate constant, **K=1/g**

Mean generation time, **g= 1/k**

The Bacterial Growth Measurement

Growth in a biological system is an orderly increase in the quantity of cellular constituents and which depends upon the ability of the cell to form new protoplasm from nutrients available in the environment. In most bacteria, growth involves increase in cell mass and number of ribosomes, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division (binary fission).

Methods for measurement of the cell mass involve both direct and indirect techniques: (Table 16.1)

- Direct physical measurement of dry weight, wet weight, or volume of cells after centrifugation.
- Direct chemical measurement of some chemical component of the cells such as total N, total protein, or total DNA content.
- Indirect measurement of chemical activity such as rate of O2 production or consumption, CO2 production or consumption, etc.
- Turbidity measurements employ a variety of instruments to determine the amount of light scattered by a suspension of cells.

Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or optical density of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve. The method is simple and nondestructive, but the sensitivity is limited to about 107 cells per ml for most bacteria. Measuring techniques involve direct counts, visually or instrumentally, and indirect viable cell counts.

Direct microscopic count

In the direct microscopic count, a counting chamber consisting of a ruled slide and a coverslip is employed. It is constructed in such a manner that the coverslip, slide, and ruled lines delimit a known volume. The

number of bacteria in a small known volume is directly counted microscopically and the number of bacteria in the larger original sample is determined by extrapolation.

The Petroff-Hausser counting chamber (commonly used in dairy industry) has small etched squares 1/20 of a millimeter (mm) by 1/20 of a mm and is 1/50 of a mm deep. The volume of one small square therefore is 1/20,000 of a cubic mm or 1/20,000,000 of a cubic centimeter (cc). There are 16 small squares in the large double-lined squares that are actually counted, making the volume of a large double-lined square 1/1,250,000 cc. The normal procedure is to count the number of bacteria in five large double-lined squares and divide by five to get the average number of bacteria per large square. This number is then multiplied by 1,250,000 since the square holds a volume of 1/1,250,000 cc, to find the total number of organisms per cc in the original sample. If the bacteria are diluted, such as by mixing the bacteria with dye before being placed in the counting chamber, then this dilution must also be considered in the final calculations.

The formula used for the direct microscopic count is

The number of bacteria per cc = The average number of bacteria per large double-lined square X The dilution factor of the large square (1,250,000) X The dilution factor of any dilutions made prior to placing the sample in the counting chamber, e.g., mixing

Electronic enumeration of cells

A Coulter counter is an apparatus for counting and sizing particles and cells. It is used, for example, for bacteria and air quality particle size distributions. The counter detects change in electrical conductance of a small aperture as fluid containing cells is drawn through. Cells, being non-conducting particles, alter the effective cross-section of the conductive channel.

It was an American inventor named Wallace H. Coulter who was responsible for the theory and design of the Coulter Counter. He first devised the theory behind its operation in 1947 while experimenting with electronics. Coulter determined that electrical charge could be used to determine the size and number of microscopic particles in a solution. This phenomenon is now known as the Coulter Principle. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. When a particle flows through one of the microchannels, it results in the electrical resistance change of the liquid filled microchannel. This resistance change can be recorded as electric current or voltage pulses, which can be correlated to size, mobility, surface charge and concentration of the particles.

Plate count method

Standard Plate Count (SPC) is a technique under this category which is commonly employed in microbiological laboratories for enumeration of bacteria .The SPC is the number of bacterial colonies that develop on a medium in a petri dish seeded with a known amount of inoculum. The number of bacteria in a

given sample is usually too great to be counted directly. However, if the sample is serially diluted (Fig.16.3) and then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies , the number of colonies can be used as a measure of the number of viable (living) cells in that known dilution. However, keep in mind that if the organism normally forms multiple cell arrangements, such as chains, the colony-forming unit may consist of a chain of bacteria rather than a single bacterium. In addition, some of the bacteria may be clumped together. Therefore, when doing the plate count technique, we generally say we are determining the number of Colony-Forming Units (CFUs) in that known dilution. By extrapolation, this number can in turn be used to calculate the number of CFUs in the original sample.

Normally, the bacterial sample is diluted by factors of 10 and plated on agar either by pour plate or spread plate technique. After incubation, the number of colonies on a dilution plate showing between 30 and 300 colonies (Fig. 16.4 and Fig. 16.5) is determined. A plate having 30-300 colonies is chosen because this range is considered statistically significant. If there are less than 30 colonies on the plate, small errors in dilution technique or the presence of a few contaminants will have a drastic effect on the final count. Likewise, if there are more than 300 colonies on the plate, there will be poor isolation and colonies will have grown together.

Generally, one wants to determine the number of CFUs per milliliter (ml) of sample. To find this, the number of colonies (on a plate having 30-300 colonies) is multiplied by the number of times the original ml of bacteria was diluted (the dilution factor of the plate counted). For example, if a plate containing a 1/1,000 dilution of the original ml of sample shows 159 colonies, then 159 represents 1/1,000 the number of CFUs present in the original ml. Therefore the number of CFUs per ml in the original sample is found by multiplying 159 x 103 (or preferably represented as 1.59 x 105) as shown in the formula below:

Advantages of the technique are its sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted. Disadvantages are

- Only living cells develop colonies that are counted;
- Clumps or chains of cells develop into a single colony;
- Colonies develop only from those organisms for which the cultural conditions are suitable for growth.

The latter makes the technique virtually useless to characterize or count the total number of bacteria in complex microbial ecosystems such as soil or the animal rumen or gastrointestinal tract. Genetic probes can be used to demonstrate the diversity and relative abundance of procaryotes in such an environment, but many species identified by genetic techniques have so far proven unculturable.

Turbidity measurement methods

When bacteria growing in a liquid medium are mixed, the culture appears turbid. This is because a bacterial culture acts as a colloidal suspension that blocks and reflects light passing through the culture.

Within limits, the light absorbed by the bacterial suspension will be directly proportional to the concentration of cells in the culture. By measuring the amount of light absorbed by a bacterial suspension, one can estimate and compare the number of bacteria present. The instrument used to measure turbidity is a spectrophotometer. It consists of a light source, a filter which allows only a single wavelength of light to pass through, the sample tube containing the bacterial suspension, and a photocell that compares the amount of light coming through the tube with the total light entering the tube. The ability of the culture to block the light can be expressed as either percent of light transmitted through the tube or the amount of light absorbed in the tube. The percent of light transmitted is inversely proportional to the bacterial concentration. The absorbance (or optical density) is directly proportional to the cell concentration.

Turbidimetric measurement is often correlated with some other method of cell count, such as the direct microscopic method or the plate count. In this way, turbidity can be used as an indirect measurement of the cell count. For example:

- Several dilutions can be made of a bacterial stock.
- A Petroff-Hausser counter can then be used to perform a direct microscopic count on each dilution.
- Then a spectrophotometer can be used to measure the absorbance of each dilution tube.
- A standard curve comparing absorbance to the number of bacteria can be made by plotting absorbance versus the number of bacteria per cc.
- Once the standard curve is completed, any dilution tube of that organism can be placed in a spectrophotometer and its absorbance read. Once the absorbance is determined, the standard curve can be used to determine the corresponding number of bacteria per cc.

The Continuous Culture of Microorganisms

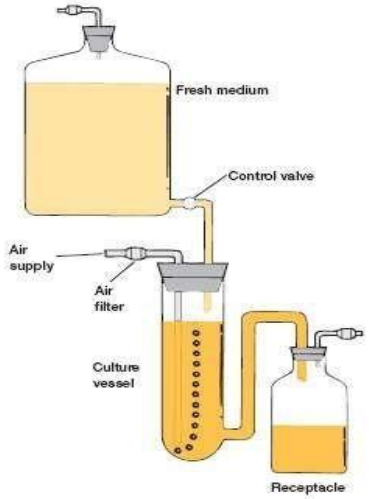
The growth of microorganisms in an open system, a system with constant environmental conditions maintained through continual provision of nutrients and removal of wastes. These conditions are met in the laboratory by a continuous culture system. A microbial population can be maintained in the exponential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

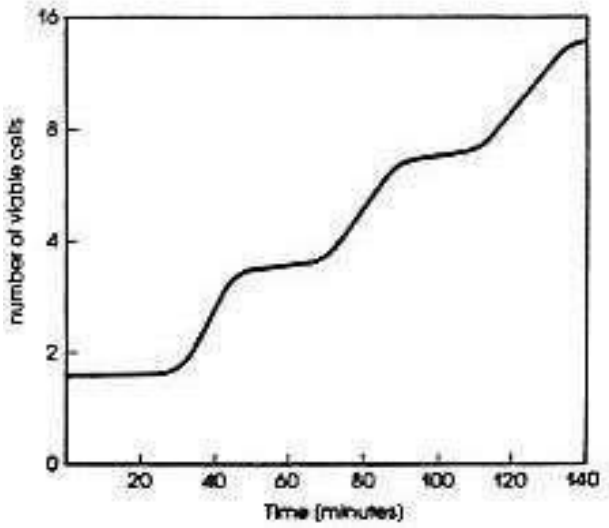
Continuous culture systems are very useful because they provide a constant supply of cells in exponential phase and growing at a known rate. They make possible the study of microbial growth at very low nutrient levels, concentrations close to those present in natural environments. These systems are essential 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.

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, is 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 o 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 mwdia 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.

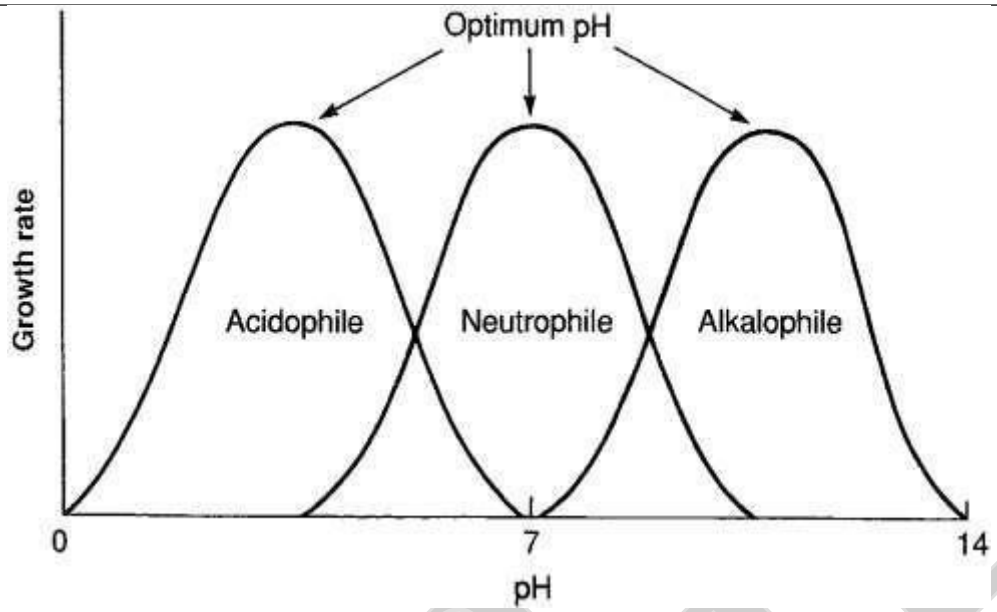
Thermophiles

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 bacteriaAnaerobic 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.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc., MB **COURSE NAME: MICROBIAL PHYSIOLOGY AND METABOLISM**

COURSE CODE: 19MBU202 **UNIT-II** **BATCH: 2019-2022**

S.no	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Diauxic growth	single growth	double growth	triple growth	slow growth	double growth
2	Synchronous culture	further no growth	cell divide slowly	All cell divide simultaneously	fast cell growth	All cell divide simultaneously
3	Synchronous culture	all cell same age	fast cell growth	further no growth	cell divide slowly	all cell same age
4	Growth occurs at a constant specific growth rate	chemostat	Batch culture method	diauxic culture	log phase	chemostat
5	Turbidostat _____	Batch culture	continuous culture	synchronous culture	Diauxic culture	continuous culture
6	Petroff Hausser counting chamber _____	measuring the cell	view the motility	view the shape	measure the size	measuring the cell
7	Measurement of bacterial cel directly	Dry weight	turbidity	nitrogen content	Petroff Hausser counting chamber	Petroff Hausser counting chamber
8	McFarland standards are used as a reference to adjust the____	Growth curve	turbidity of bacterial suspensions	medium	dilution	turbidity of bacterial suspensions
9	Membrane filter technique	measure the bacterial cell	filtration of all microbes	purification	killing the microbes	measure the bacterial cell
10	_____can survive in all sorts of extremely hostile environment.	mesophiles	thermophiles	Extremophiles	Halophiles	Extremophiles
11	_____Can survive in hotspring	Themophiles	mesophiles	Extremophiles	Halophiles	Themophiles
12	*Thermus thermophilus is example for* _____	Themophiles	mesophiles	Extremophiles	Halophiles	Themophiles
13	Organisms that live in environment with very high concentration of salt	mesophiles	thermophiles	Extremophiles	Halophiles	Halophiles

14	Time between two consecutive generation is	Lag phase	log phase	decline	generation time	generation time
15	Microbial growth increases the number of cells and the .	Biomass	Rate	Doubling time	Structure	Rate
16	Reproduction of bacterial cells take place by .	Pollination	Binary fission	Mitosis	Meiosis	Binary fission
17	Doubling time is otherwise called as .	Growth curve	Growth rate	Generation time	Generation period	Generation time
18	The generation time is as short as 20 minutes under optimal condition in .	Klebsiella	Proteus	Salmonella	E. coli	E. coli
19	Methanococcus has a doubling time of _____ minutes	20	10	30	50	20
20	_____ are responsible for the biosynthesis of proteins	Nucleus	Ribosome	DNA	Sulphur	Ribosome
21	In the atmosphere the availability of water is expressed as .	Relative humidity	Xerotolerant	Osmosis	Water activity	Relative humidity
22	The typical growth curve of a bacterial culture begins with the .	Log phase	Lag phase	Death phase	Exponential growth phase	Lag phase
23	The growth phase at which there is no net increase in bacterial cell numbers is called .	Stationary phase	Exponential growth phase	Lag phase	Death phase	Stationary phase
24	The number of viable cells begining to decline signally the onset of the _____.	Log phase	Lag phase	Death phase	Exponential growth phase	Death phase

25	_____ measures the turbidity of the culture in the growth vessel.	Synchronous method	Batch culture method	Turbidostat	Thermostat	Turbidostat
26	The growth rate of bacteria in nature can be estimated by using .	Synchronous method	Batch culture method	Turbidostat	Chemostat	Chemostat
27	_____of bacteria occurs when all cells divide at the same time.	Synchronous culture	Batch culture method	Turbidostat culture	Thermostat culture	Synchronous culture
28	Some bacteria known as _____grow only at temperature near their optimal temperature.	Euthermal	Stenothermal	thermophiles	Capnophiles	Stenothermal
29	The alkaliphiles occupy the _____	High end of pH spectrum	Low end of pH spectrum	neare to neutral Ph	neutral Ph	High end of pH spectrum
30	Proton motive force used to synthesis the	ADP	NADH	NADPH2	ATP	ATP
31	Microorganisms which can thrive and grow in harsh conditions are often called	Halophiles	Halophiles	Extremophiles	Mesophiles	Extremophiles
32	_____is a hydrogen ion activity	Temperature	aeration	osmotic pressure	Ph	Ph
33	Extreme alkalophiles maintain their internal pH closer to _____	alkalinity	acidity	high alkalinity	neutrality	neutrality
34	Which of the following media would not be used to culture aerobes?	selective media	differential media	complex media	reducing media	reducing media
35	Which one of the following temperature would most likely kill a mesophiles?	50	9	60	37	60

36	The term trace elements refers to _____	thye elements CHONPS	vitamines	nitogen, phosphorous	small mineral requirements	small mineral requirements
37	Diauxic growth by utilizing_____in growth media	two sugar	single sugar	aminoacid	vitamine	two sugar
38	High pressure denature the _____in vegetative cell	lipid	cell wall	protein	membrane	protein
39	Endospores can resist	low salinity	salinity	water activity	Desiccation	Desiccation
40	Microorganism in high concentration of salts and sugars undergo	damage	osmosis	cell lysis	plasmolysis	plasmolysis
41	The effect of radiation depend on _____	speed	intensity	dose	exposure	intensity
42	_____is a nonionizing radiation	electron beam	UV	Gamma	X ray	UV
42	The growth curve generated by an organism which has two growth peaks	Diauxic growth	generation time	continuous growth	batch culture	Diauxic growth
43	Microbial population can be maintained in a state of exponential growth over a long period of time by	Batch culture	Continuous culture	Synchronous culture	Pure culture	Continuous culture
44	Which of the following is used for continuous culture ?	Role tube	Chemostat	Thermostat	Conical flask	Conical flask
45	At which of the following temperature is the growth rate greatest ?	Optimum	Maximum	Minimum	Cardinal	Optimum
46	A bacterial culture began with cells and ended with 128 cells how many generations did the	64	32	6	5	6

	population go through?					
47	Microbial cultures composed of cells that are all the same stage of the cell cycle are called	Axenic culture	Pure culture	Mixed culture	Synchronous culture	Synchronous culture
48	Microbial population can be maintained in a state of exponential growth over a long period of time by	Batch culture	Continuous culture	Synchronous culture	Pure culture	Continuous culture
49	The time required for the doubling of cell mass is known as _____	Doubling time	Generation time	Generation gap	Developing time	Doubling time
50	The formula for calculating total number of bacterial cell after 'n' number of generations is	$N = 1x2^n$	$N = 2x2^n$	$N = 1x1^n$	$N = 2x1^n$	N = 1x2n
51	The time required for the doubling of cell mass is known as _____	Doubling time	Generation time	Generation gap	Developing time	Doubling time
52	Growth rate is the reciprocal of _____	Doubling time	Cell division	Binary fission	Generation time	Cell division
53	In_____phase rate of multiplication of bacteria increases with time.	Lag	Log	Stationary	Decline	Log
54	If a cell concentration is periodically measured in a fresh medium _____describes the change in cell number against time.	Growth curve	Growth rate	Generation time	Generation period	Growth curve
55	The log growth phase is also called as .	Stationary phase	Exponential growth phase	Lag phase	Death phase	Exponential growth phase
56	Too much of acid or base _____	promote cellular activity	disturb the cellular activity	enhance the cell growth	activate the metabolism	disturb the cellular activity

57	The growth is modified by controlling and monitoring the turbidity of the culture is called _____	Synchronous method	Batch culture method	Turbidostat	Thermostat	Turbidostat
58	A few organisms can reduce elemental nitrogen to ammonia and this process of nitrogen assimilation is known as	Biological nitrogen fixation	Biological nitrite fixation	Biological ammonia fixation	Ammonification	Biological nitrite fixation
59	The_____of the microorganism is the time that it takes for the cell to reproduce.	Growth curve	Growth amount	Growth rate	Biomass	Growth rate

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.

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 bisphosphatase 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.

Glycolysis Pathway

Glycolysis is the first step in the breakdown of glucose to extract energy for cellular metabolism. Glycolysis consists of an energy-requiring phase followed by an energy-releasing phase.

Glycolysis is the sequence of 10 enzyme catalyzed reactions that converts glucose into pyruvate with the simultaneous production of ATP.

The overall reaction of glycolysis which occurs in the cytoplasm is represented simply as:

$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ P$ —> 2 pyruvic acid, $(CH_3(C=O)COOH + 2\ ATP + 2\ NADH + 2\ H^+$

Steps of Glycolysis

Glycolysis is an extramitochondrial pathway and is carried by a group of eleven enzymes. Glucose is converted to pyruvate in 10 steps by glycolysis. The glycolytic patway can be divided into two phases:

Preparatory Phase :

This phase is also called glucose activation phase. In the preparatory phase of glycolysis, two molecules of ATP are invested and the hexose chain is cleaved into two triose phosphates. During this, phosphorylation of glucose and it's conversion to glyceraldehyde-3-phosphate take place. The steps 1, 2, 3, 4 and 5 together are called as the preparatory phase.

Payoff Phase :

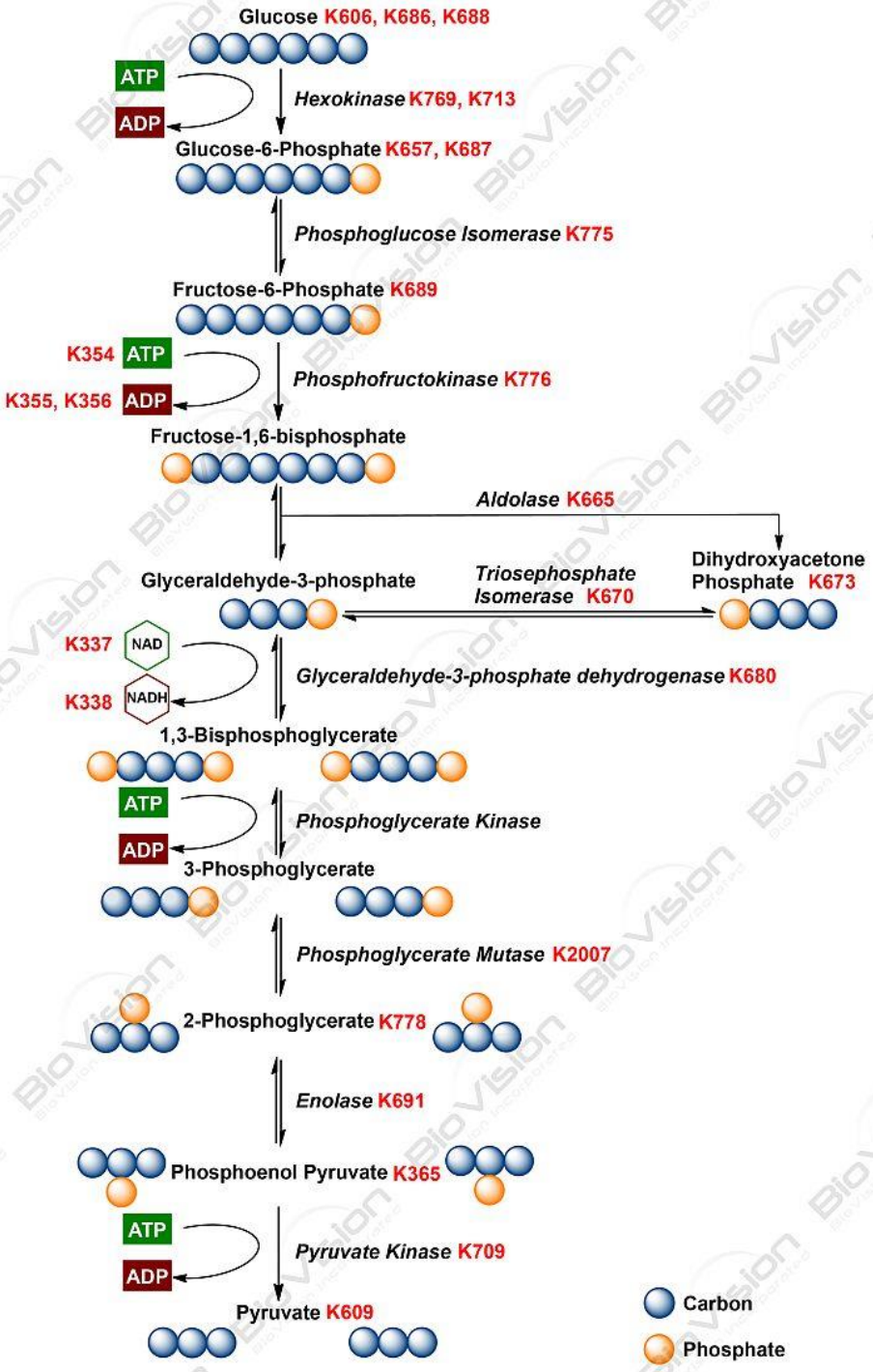
This phase is also called energy extraction phase. During this phase, conversion of glyceraldehyde-3-phophate to pyruvate and the coupled formation of ATP take place. Because Glucose is split to yield two molecules of D-Glyceraldehyde-3-phosphate, each step in the payoff phase occurs twice per molecule of glucose. The steps after 5 constitute payoff phase.

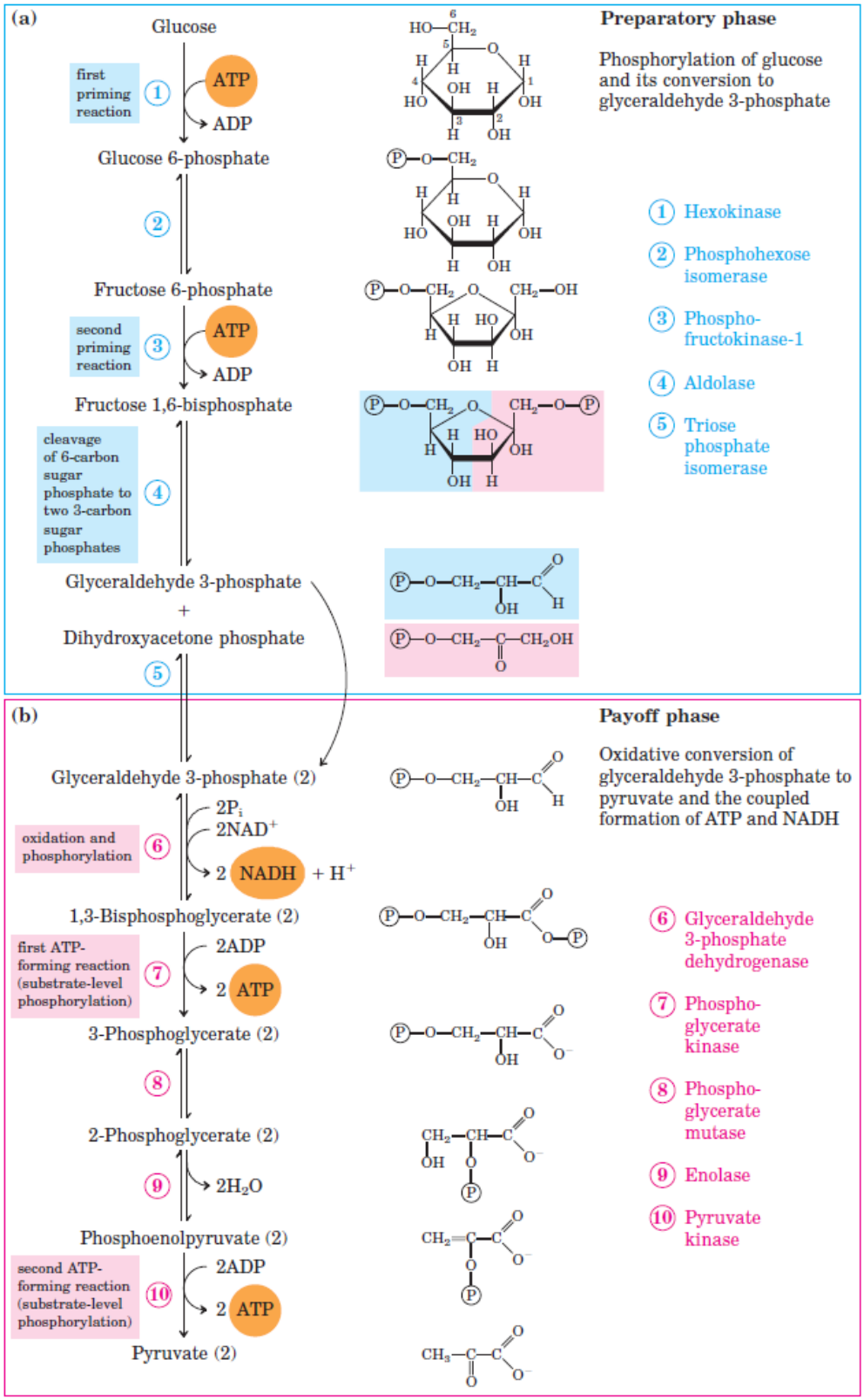
Steps of Glycolysis

1. The first step in glycolysis is the conversion of D-glucose into glucose-6-phosphate. The enzyme that catalyzes this reaction is hexokinase.
2. The second reaction of glycolysis is the rearrangement of glucose 6-phosphate (G6P) into fructose 6-phosphate (F6P) by glucose phosphate isomerase (Phosphoglucose Isomerase).
3. Phosphofructokinase, with magnesium as a cofactor, changes fructose 6-phosphate into fructose 1,6-bisphosphate.
4. The enzyme Aldolase splits fructose 1, 6-bisphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP).
5. The enzyme triophosphate isomerase rapidly inter- converts the molecules dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). Glyceraldehyde phosphate is removed / used in next step of Glycolysis.
6. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dehydrogenates and adds an inorganic phosphate to glyceraldehyde 3-phosphate, producing 1,3-bisphosphoglycerate.
7. Phosphoglycerate kinase transfers a phosphate group from 1,3-bisphosphoglycerate to ADP to form ATP and 3-phosphoglycerate.
8. The enzyme phosphoglycero mutase relocates the P from 3- phosphoglycerate from the 3rd carbon to the 2nd carbon to form 2-phosphoglycerate.
9. The enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvic acid (PEP).

10. The enzyme pyruvate kinase transfers a P from phosphoenolpyruvate (PEP) to ADP to form pyruvic acid and ATP Result in step 10.

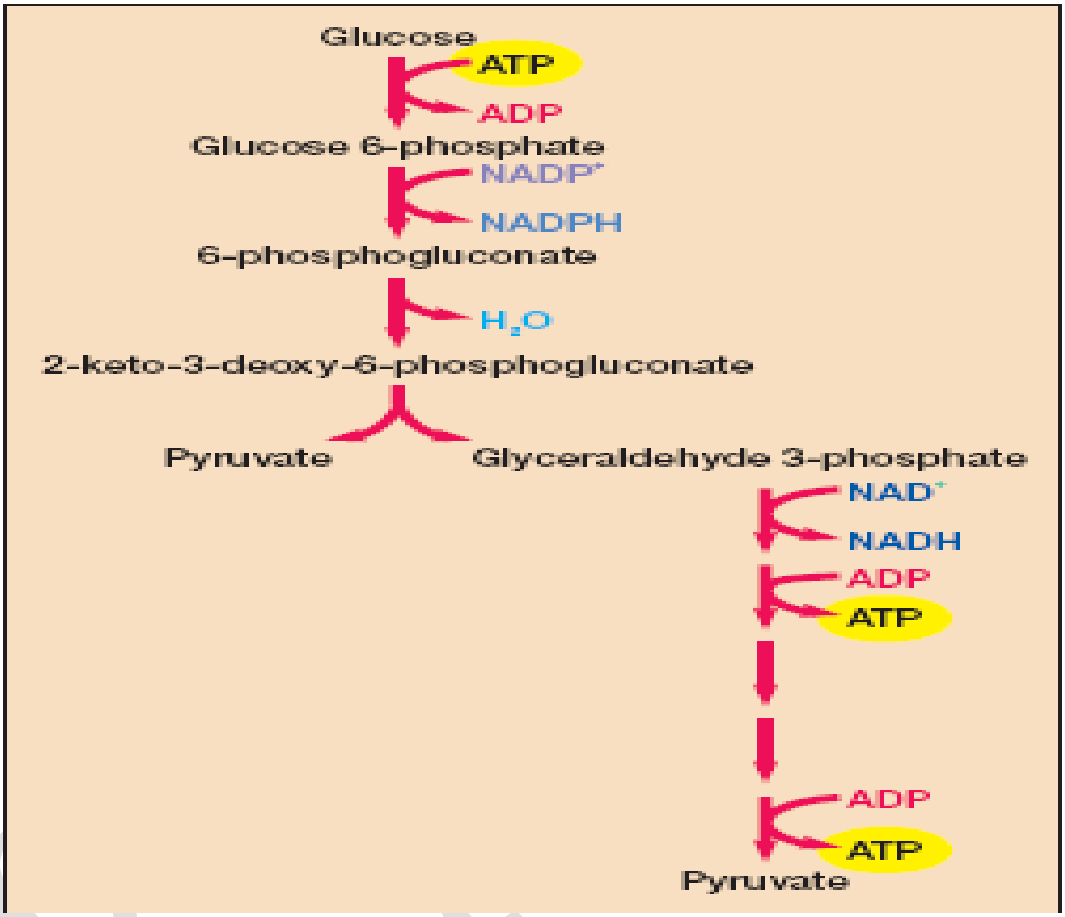
Although 2 ATP molecules are used in steps 1-3, 2 ATP molecules are generated in step 7 and 2 more in step 10. This gives a total of 4 ATP molecules produced. If you subtract the 2 ATP molecules used in steps 1-3 from the 4 generated at the end of step 10, you end up with a net total of 2 ATP molecules produced.




(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase
(b)
Glyceraldehyde 3-phosphate (2)
oxidation and phosphorylation
6
2Pi
2NAD+
2 NADH + H+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H2O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase

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



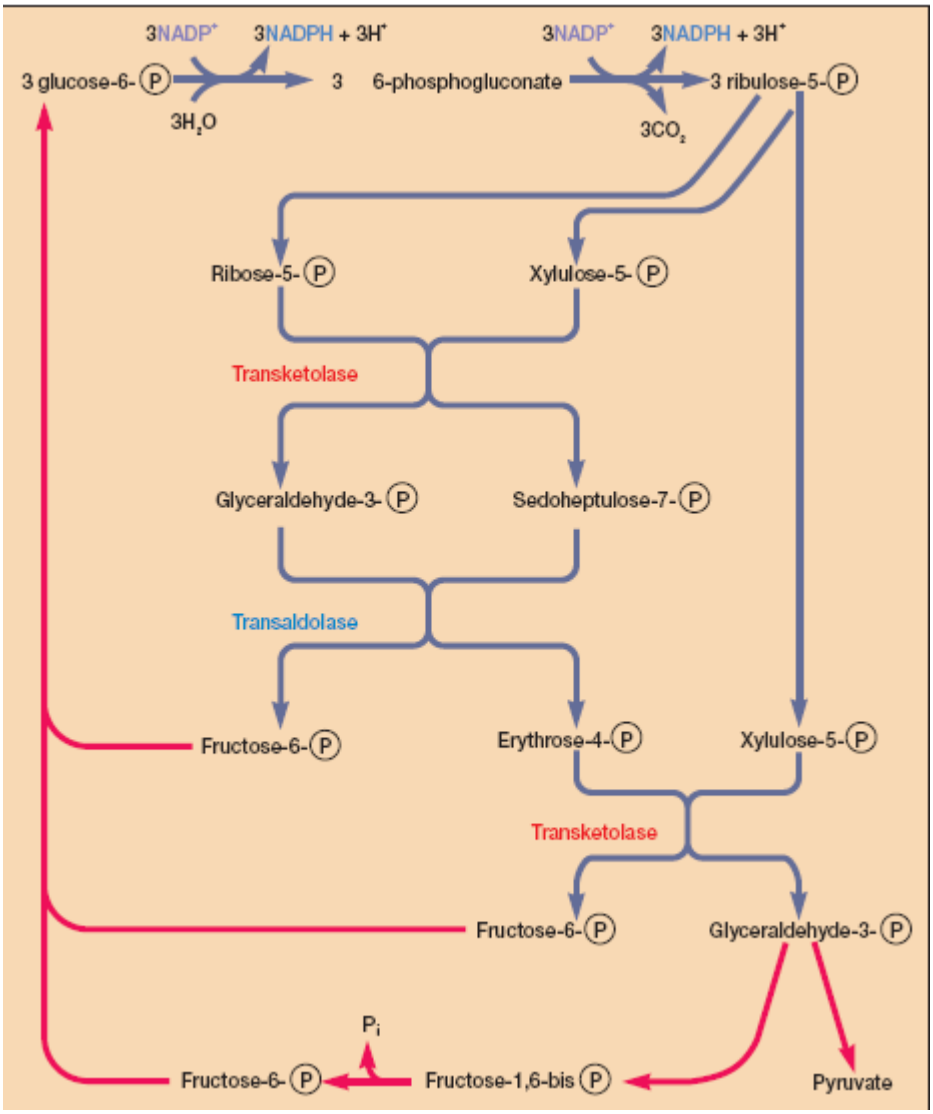
glucose 6-phosphate and 6-phosphogluconate.

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 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.

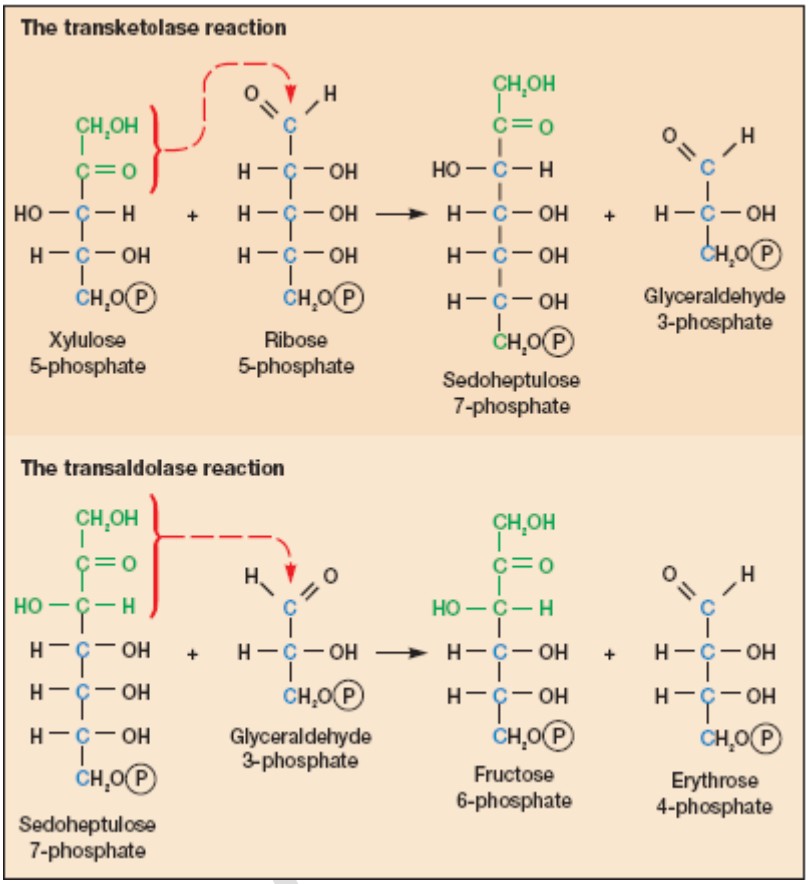


The conversion of three glucose 6-phosphate molecules to two fructose 6-phosphates and a glyceraldehyde 3-phosphate is traced. The fructose 6-phosphates are changed back to glucose 6-phosphate. The glyceraldehyde 3-phosphate can be converted to pyruvate or combined with a molecule of dihydroxyacetone 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.

$$3\ \text{glucose 6-phosphate} + 6NADP^+ + 3H_2O \longrightarrow 2\ \text{fructose 6-phosphate} + \text{glyceraldehyde 3-phosphate} + 3CO_2 + 6NADPH + 6H^+$$

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.

$$\text{Glucose 6-phosphate} + 12NADP^+ + 7H_2O \longrightarrow 6CO_2 + 12NADPH + 12H^+ + P_i$$

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.

Citric Acid Cycle (Krebs Cycle)

Like the conversion of pyruvate to acetyl CoA, the citric acid cycle takes place in the matrix of the mitochondria. Almost all of the enzymes of the citric acid cycle are soluble, with the single exception of the enzyme succinate dehydrogenase, which is embedded in the inner membrane of the mitochondrion. Unlike glycolysis, the citric acid cycle is a closed loop: the last part of the pathway regenerates the compound used in the first step. The eight steps of the cycle are a series of redox, dehydration, hydration, and decarboxylation reactions that produce two carbon dioxide molecules, one GTP/ATP, and reduced forms of NADH and FADH2. This is considered an aerobic pathway because the NADH and FADH2 produced must transfer their electrons to the next pathway in the system, which will use oxygen. If this transfer does not occur, the oxidation steps of the citric acid cycle also do not occur. Note that the citric acid cycle produces very little ATP directly and does not directly consume oxygen.

Steps in the Citric Acid Cycle

Step 1. The first step is a condensation step, combining the two-carbon acetyl group (from acetyl CoA) with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate. CoA is bound to a sulfhydryl group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible because it is highly exergonic. The rate of this reaction is controlled by negative feedback and the amount of ATP available. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases.

Step 2. Citrate loses one water molecule and gains another as citrate is converted into its isomer, isocitrate.

Steps 3 and 4. In step three, isocitrate is oxidized, producing a five-carbon molecule, α-ketoglutarate, together with a molecule of CO_2 and two electrons, which reduce NAD+ to NADH. This step is also regulated by negative feedback from ATP and NADH and by a positive effect of ADP. Steps three and four are both oxidation and decarboxylation steps, which release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. α-Ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds the succinyl group to form succinyl CoA. The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.

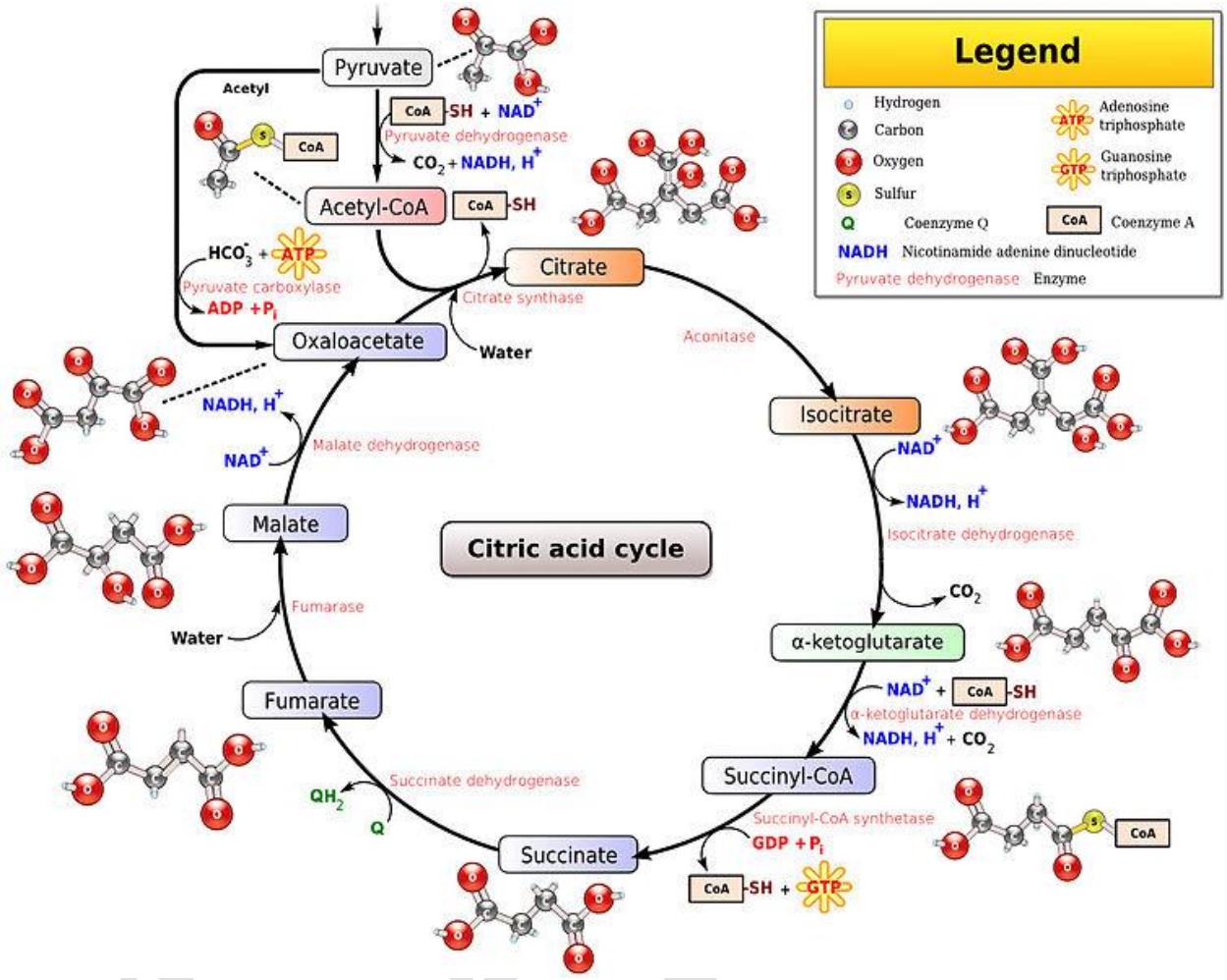
Step 5. A phosphate group is substituted for coenzyme A, and a high- energy bond is formed. This energy is used in substrate-level phosphorylation (during the conversion of the succinyl group to succinate) to form either guanine triphosphate (GTP) or ATP. There are two forms of the enzyme, called isoenzymes, for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. In particular, protein synthesis primarily uses GTP.

Step 6. Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. The energy contained in the electrons of these atoms is insufficient to reduce NAD^+ but adequate to reduce FAD. Unlike NADH, this carrier remains attached to the enzyme and transfers the electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion.

Step 7. Water is added to fumarate during step seven, and malate is produced. The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced.

Products of the Citric Acid Cycle

Two carbon atoms come into the citric acid cycle from each acetyl group, representing four out of the six carbons of one glucose molecule. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not necessarily contain the most recently-added carbon atoms. The two acetyl carbon atoms will eventually be released on later turns of the cycle; thus, all six carbon atoms from the original glucose molecule are eventually incorporated into carbon dioxide. Each turn of the cycle forms three NADH molecules and one $FADH_2$ molecule. These carriers will connect with the last portion of aerobic respiration to produce ATP molecules. One GTP or ATP is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is amphibolic (both catabolic and anabolic).

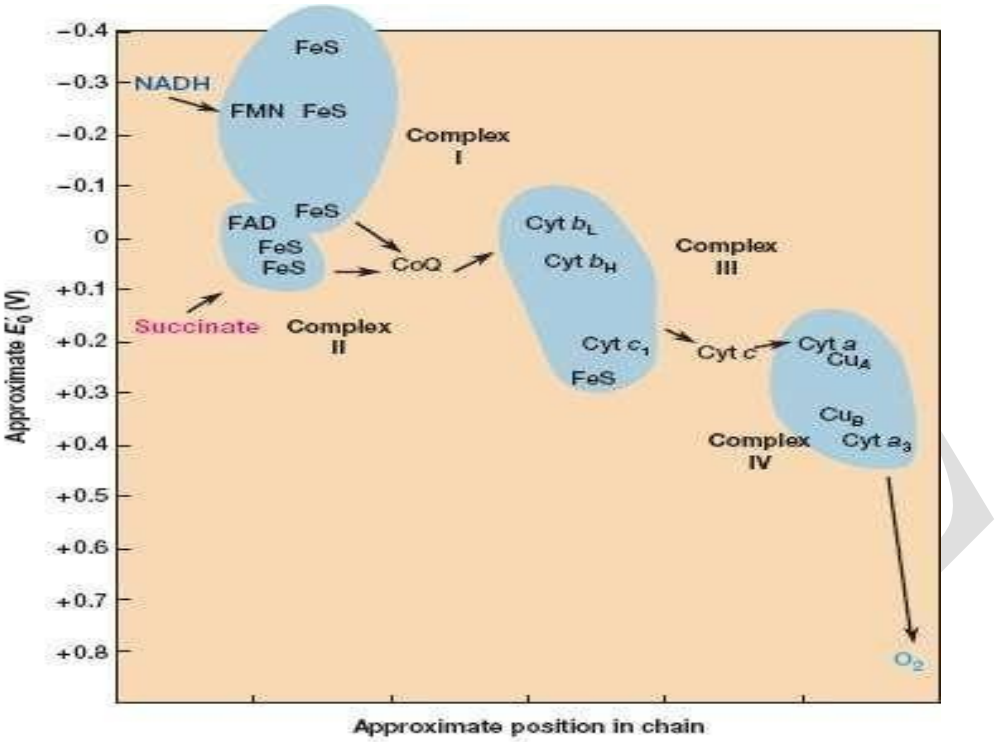


Electron Transport and Oxidative Phosphorylation

Little ATP has been synthesized up to this point. Only the equivalent of four ATP molecules is directly synthesized when oneglucose is oxidized to six CO_2 molecules by way of glycolysis and the TCA cycle. Most ATP generated comes from the oxidation of NADH and $FADH_2$ in the electron transport chain. The mitochondrial electron transport chain will be examined first because it has been so well studied. Then we will turn to bacterial chains, and finish with a discussion of ATP synthesis.

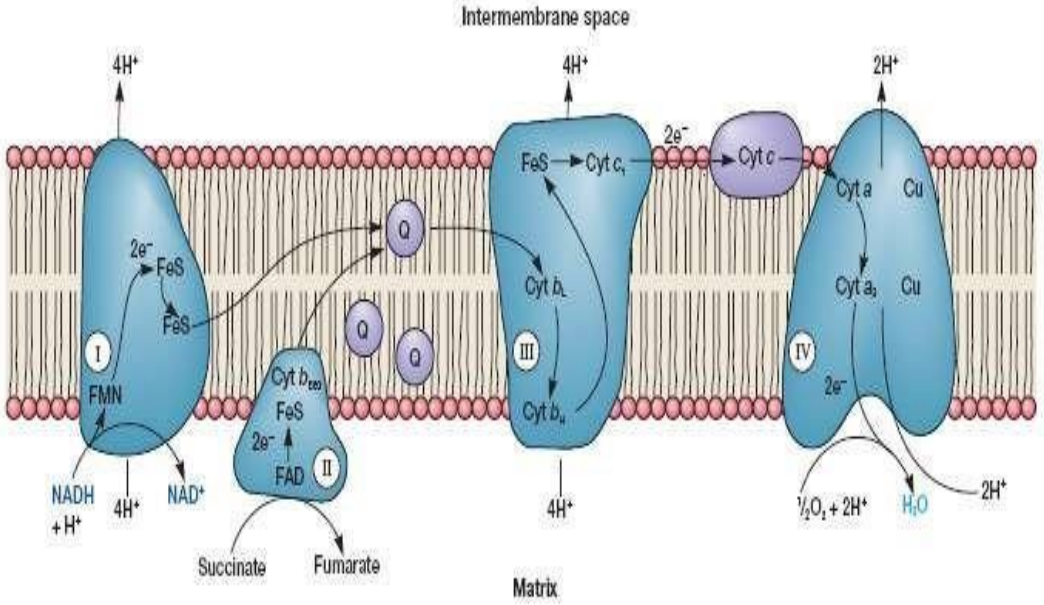
The Electron Transport Chain

The mitochondrial **electron transport chain** is composed of a series of electron carriers that operate together to transfer electrons from donors, like NADH and $FADH_2$, to acceptors, such as O_2.



The Mitochondrial Electron Transport Chain

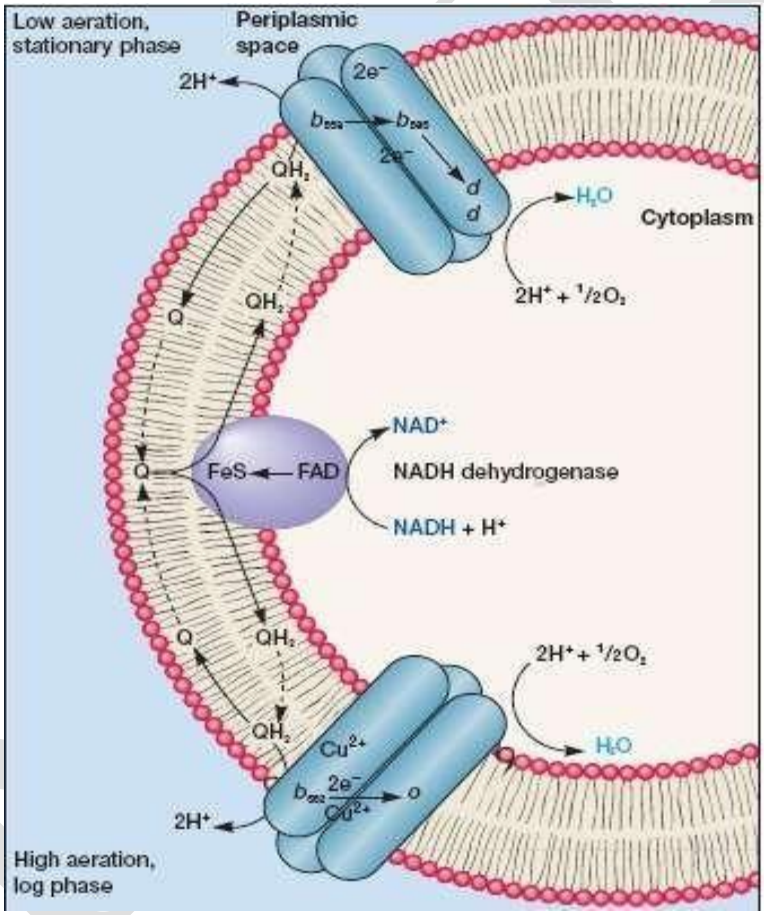
The electrons flow from carriers with more negative reduction potentials to those with more positive potentials and eventually combine with O_2 and H^+ to form water. The electrons move down this potential gradient much like water flowing down a series of rapids. The difference in reduction potentials 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 O_2. This is the same thing as saying that the phosphorus to oxygen (P/O) ratio is equal to 3. Because electrons from $FADH_2$ only pass two oxidative phosphorylation points, the maximum P/O ratio for $FADH_2$ 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*

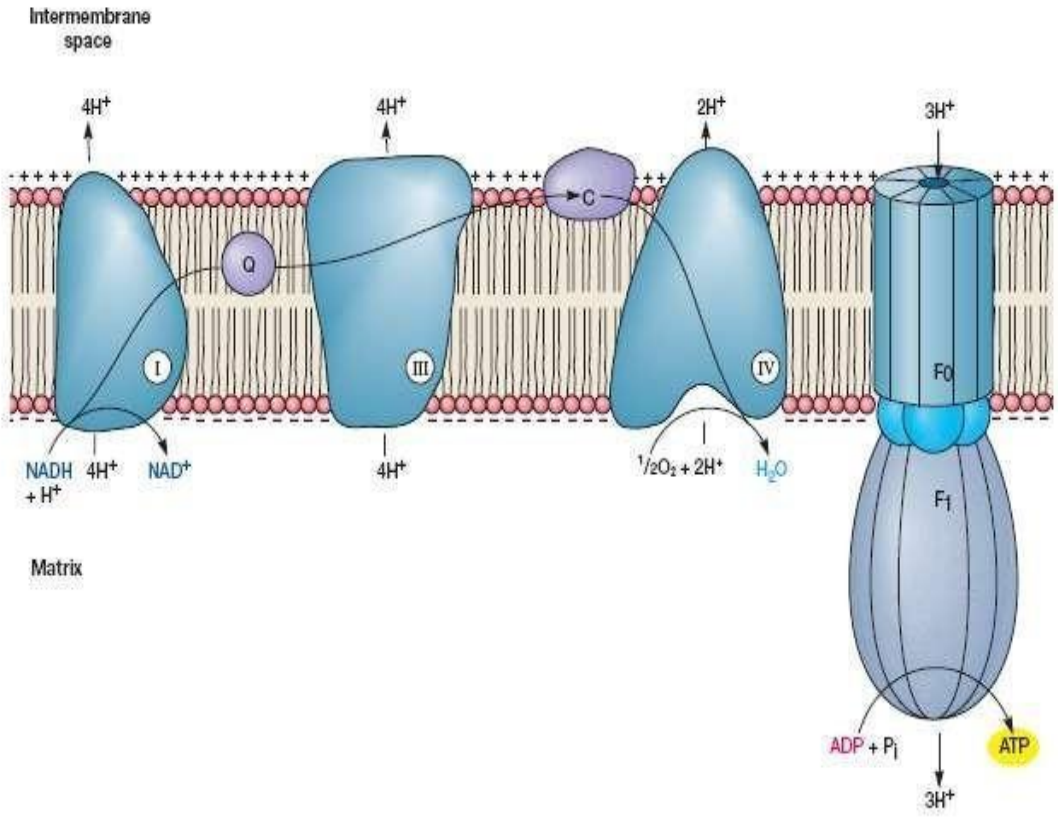
Although it transports electrons from NADH to acceptors and moves protons across the plasma membrane, the *E. coli* chain is quite different from the mitochondrial chain. For example, it is branched and contains a quite different array of cytochromes. Coenzyme Q or ubiquinol donates electrons to both 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.



Chemiosmsis

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.

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 Kreb'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.

Give an account on uncouplers and inhibitors.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc., MB **COURSE NAME: MICROBIAL PHYSIOLOGY AND METABOLISM**

COURSE CODE: 19MBU202 **UNIT-III** **BATCH: 2019-2022**

S.no	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Glycolysis is dissimilatory pathway that results in the breakdown of a molecule of glucose into two molecules of __	galactose	pyruvate	oxaloacetate	malate	pyruvate
2	In Cyclic photophosphorylation, electron enters	photosystem I	photosystem II	EMP pathway	HMP pathway	EMP pathway
3	Photosystem I only reduce	NADPH	NADP	NADPH2	NADH	NADP
4	The herbicide inhibits reduction of_____ activity in oxidative phosphorylation	Cytochrome	Auxochrome	Dichrome	Methanoctrome	Methanoctrome
5	Precursor for purine biosynthesis	PRPP		PPR	RPRR	PRPP
6	Nitrogen fixation reduces	Nicotin amide nucleotides	Biotin amide nucleotides	Biotin by-products	Nucleotides	Nucleotides
7	Intial carrier which accepts electrons in electron transport chain is	Ubiquinone.	Monoquinone	Cytochrome c	Cytochrome f	Ubiquinone.
8	_____is needed to drive the formation of ATP.	PMF	UTP	GTP	GDP	PMF
9	The electrons are transferred unidirectionally in ____pathway.	Z pathway	Glyoxalate pathway	Non-cyclic pathway	EMP pathway	Non-cyclic pathway
10	Electrons can flow cyclically in_____.	Photosystem I	Photosystem II	Photosystem I & II	None	Photosystem I
11	The photosystems has its own_.	Z pathway	PMF	Photoreaction center	Non-cyclic pathway	Photoreaction center
12	Photosystem I and II linked into unified pathway called_____.	Non-cyclic pathway	Z pathway	Both a and b	Photoreaction center	Z pathway
13	Converts glucose into pyruvate	glycolysis	TCA cycle	ED pathway	HMP	glycolysis
14	The ED pathway is generally found in	Pseudomonas	Streptococcus	Vibrio	Proteus	Pseudomonas
15	The number of ATP generated during ED pathway is .	2	1	3	4	1

16	The glycolytic pathway degrades one glucose to pyruvates.	1	3	5	2	2
17	The pentose phosphate pathway is otherwise called as .	ED pathway	TCA cycle	HMP pathway	Krebs cycle	HMP pathway
18	_____ enzymes catalyse the transfer of 2-C ketol groups in HMPpathway.	transaldolase	transketolase	epimerase	kinase	transketolase
19	The EMP pathway occurs in the_____ of procaryotes and eukaryotes.	ribosomes	nucleus	plasma membrane	cytoplasmic matrix	cytoplasmic matrix
20	The process in which radient energy is used to generate ATP is called	Photophosphorylation	Oxidative phosphorylation	Substrate level phosphorylation	Fermentation	Photophosphorylation
21	The enzyme involved in the conversion of glucose to glucose-6 phosphate in EMP pathway is .	aldolase	Hexokinase	enolase	kinase	Hexokinase
22	The conversion of fructose 6 phosphate to fructose 1,6-bisphosphate in EMP pathway is catalysed by enzyme.	aldolase	Isomerase	Phosphofructokinase	Enolase	Phosphofructokinase
23	The formation of ATP in EMP pathway is carried out by reaction.	Aerobic respiration	Substrate level phosphorylation	Oxidative phosphorylation	Fermentation	Substrate level phosphorylation
24	_____enzyme of EMP pathway was lacking in ED pathway.	6-phosphofructokinase	dehydrogenase	kinase	lyase	6-phosphofructokinase
25	Glycolytic pathway generate ____by substrate –level phosphorylation	ATP	ADP	UTP	UDP	ATP
26	In Alcoholic fermentation, Pyruvate is converted to	Ethanol & O2	Ethanol & Co2	Methanol &Co2	Methanol &O2	Ethanol & Co2
27	Purine base present in an ATP molecule is	Cytosine	Thrionone	Quanine	Adenine	Adenine
28	_____is the most common pathway for glucose degradation to pyruvate.	HMP pathway	ED pathway	EMP pathway	TCA cycle	EMP pathway

29	The addition of phosphate group to a compound is called ___	Carboxylation	Hydroxylation	Phosphorylation	Pyrophosphorylation	Phosphorylation
30	The compound that supplies electron for an electron-transport system is called _____	Electron donor	Elector acceptor	Proton	Neutron	Electron donor
31	In an electron transport chain_ions are pumped across the membrane and accumulate on one side.	Nitrogen	Carbon	Hydrogen	Phosphate	Phosphate
32	ATP is hydrolyzed to give	Force	Action	Metabolism	Energy	Energy
33	Dissimilation is _____of nutrients during which energy is released	Break up	Break down	formation	reduction	Break down
34	Glycolysis is dissimilatory pathway that results in the breakdown of a molecule of glucose into two molecules of __	Pyruvic acid	Lactic acid	Nucleic acid	Acidic acid	Pyruvic acid
35	Aerobic respiration the terminal electron receptor is ___	O_2	N_2	H_2	CO_2	O_2
36	Metabolism by glycolysis gives a net yield of _____ATP molecules.	4	2	3	1	1
37	In comparing the efficiency of fermentation versus respiration with regard to ATP yield___is the more efficient process.	Respiration	Fermentation	Oxidation	Carboxylation	Respiration
38	Energy production in anaerobes is not by	TCP cycle	EMP pathway	Fermentation	Pentose phosphate shunt	Pentose phosphate shunt
39	Sulphur is needed for the biosynthesis of aminoacids such as	Methionine	Cysteine	Cystine	Valine	Methionine
40	Which of the following statement is correct?	Dissimilation of nutrients provides the building blocks for the synthesis of cell constituents.	Energy is not required for the repair of damage	Synthesis of cell constituents is an energy liberating process	Dissimilation is an energy requiring process	Dissimilation of nutrients provides the building blocks for the synthesis of

						cell constituents
41	A molecule that loses a hydrogen atom is said to have been oxidized, because a hydrogen atom contains____	An ion	A proton	A neutron	An electron	An electron
42	Proton motive force can be used to synthesise	Flagella	ATP	Protein	Hydrogen atom	ATP
42	During glycolysis which type of phosphorylation generates ATP?	Photophosphorylation	Oxidative phosphorylation	Substrate level phosphorylation	Cyclic phospho rylation	Substrate level phosphorylati
43	Which of the following biochemical pathways occur only in microorganisms?	Embden-Meyerhoff pathway	Pentose Phosphate pathway	Glycolytic pathway	Entner-Doudoroff pathway	Glycolytic pathway
44	Fermentation yields ATP per substrate molecule than respiration.	More	Equal	Less	Abundant	Less
45	Which of the following require a high concentration of sodium	Cyanobacteria	Marine bacteria	Photosynthetic bacteria	Iron bacteria	Marine bacteria
46	The synthesis of ATP in fermentation is due to	Oxidative phosphorylation	Kreb's cycle	EMP pathway	Sustrate level phospho rylation	Sustrate level phosphorylatio
47	pathway is used in homolactic acid fermentation.	ED pathway	EMP pathway	Kreb's cycle	Fermentation	EMP pathway
48	The ethanol and carbon dioxide produced during heterolactic acid fermentation comes from of the pathway.	Glycolytic portion	Oxidative portion	fermentative portion	Kreb's portion	Glycolytic portion
49	The process of break down a__is called glycolysis	Phosphates	CO2	Sugar	Nitrates	sugar
50	APS stands for _____.	Active protein surface	Ammonium phosphate and sulfur	Adenosine phosphosulfate	Adenosine potassium sulfur	Active protein surface

51	The APS is phosphorylated by a second ATP molecule to form_.	PAPS	ATP	ADP	AMP	PAPS
52	_____serves as the precurssor for thyamidine triphosphate which occurs in DNA.	UTP	CTP	AMP	PAPS	UTP
53	The conversion of Glucose-6 phosphate to fructose 1,6 bisphosphate catalysed by ____.	Phosphofructo kinase	Phosphohexo kinase	Lipase	Epimerase	Lipase
54	All living organisms use _____as central currency of energy.	ADP	ATP	AMP	FAD	AMP
55	In _____pathway, substances are broken down into smaller molecules.	Anabolic	Catabolic	Biological	Glycolytic	Catabolic
56	_____is a readily available intermediate of glycolysis.	Acetoin	Acetyl CoA	Dihydroxyaceton e phosphate	Flavoprotein	Dihydroxyaceton e phosphate
57	In Non Cyclic photophosphorylation, electron enters	photosystem I	photosystem II	EMP pathway	HMP pathway	photosystem I
58	Molecular weight of ferridoxin is _____dalton	12,000	10,000	8,000	5,000	12,000
59	_____ is an acidic protein	Plastocyanin	phytocyanin	Plastobilin	Phycobilin	Plastocyanin

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 (homofermentative and heterofermentative pathways), concept of linear and branched fermentation pathways.

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.

$$NO_3^- + 2e^- + 2H^+ \longrightarrow NO_2^- + H_2O$$

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.

$$2NO_3^- + 10e^- + 12H^+ \longrightarrow N_2 + 6H_2O$$

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.

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

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 *d*1 (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 CO_2 or carbonates as a terminal electron acceptor are called methanogens because they reduce CO_2 to methane. Sulfate also can act as the final acceptor in bacteria such as *Desulfovibrio*. It is reduced to sulfide (S_2 or H_2S), and eight electrons are accepted.

$$SO_4^{2-} + 8e^- + 8H^+ \longrightarrow S^{2-} + 4H_2O$$

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.

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: gylcolysis 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.

$$C6H12O6 \rightarrow 2\ CH3CHOHCOOH$$

Heterolactic Fermentation

This involves the use of pyruvate to produce lactic acid, ethanol, and carbon dioxide as byproducts,

$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$

under the aid of the enzymes lactate dehydrogenase and pyruvate decarboxylase.

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.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc., MB **COURSE NAME: MICROBIAL PHYSIOLOGY AND METABOLISM**

COURSE CODE: 19MBU202 **UNIT-IV** **BATCH: 2019-2022**

S.no	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	The biosynthesis of lipopolysaccharides occur at	Nuclear membrane	Plasma membrane	Cytoplasmic membrane	Periplasmic space	Cytoplasmic membrane
2	The peptidoglycan layer of Gram negative bacteria is located in the _____space	Triplasmic	Metaplasmic	Periplasmic	Megaplasmic	Periplasmic
3	EMP pathway otherwise called as	glycolysis	TCA cycle	ED pathway	HMP	glycolysis
4	___are components of nucleic acid	aminoacid	protein	carbon	purine	purine
5	Pyrimidine is the components of	cell wall	nucleic acid	Cytoplasmic membrane	Mitochondria	nucleic acid
6	PRPP is a_____donor	Glucose	fructose	ribose	mannose	ribose
7	Purines are one of two families of nitrogen-containing molecules called _	nitrogenous bases	protein bases	sugar bases	Nucleotides	nitrogenous bases
8	Gram positive Cell wall assembly is catalyzed by _	Penicillin binding protein	protein	ferridoxin	Cofactors	Penicillin binding protein
9	Synthesis of lipoteichoic acid is occur on the surface of the	membrane	cytoplasmic membrane	nucleus	cell membrane	cytoplasmic membrane
10	R5P normally derived from___	Pentose phosphate pathway	EMP pathway	ED pathway	TCA cycle	Pentose phosphate pathway
11	Gram negative bacteria must transport _____	murein	Lipopolysaccharide	Murein precursors	pseudomurein	Lipopolysaccharide
12	Attachment of the completed teichuronic acid to peptidoglycan apparently occurs by	phosphodiester linkage	diester bond	ester bond	phosphoric linkage	phosphodiester linkage
13	_____Is generally accepted as the energy provider for transport of lipopolysaccharide precursors across the membrane	proton force	Proton motive force	transport chain	electron transport chain	Proton motive force

14	_____between murein strands, the gram positive wall is inherently stronger than the gram negative wall.	peptido glycan content	Lipopolysaccharide	murein precursors	Cofactors	peptidoglycan content
15	Covertion of acid into aminoacid is called _____	amination	transcription	translation	transaminatio n	amination
16	Amine group comes from preexisting aminoacid is called	amination	transcription	translation	transaminatio n	transamination
17	The joining of aminoacids to form proteins _____	require ATP	loss energy	loss ATP	require GTP	require ATP
18	peptidoglycan monomers are synthesised in	prenol	cytoplasam	cell	cytosol	cytosol
19	Peptidoglycan layer is_in gram positive than Gram negative bacteria	larger	lesser	thicker	smaller	thicker
20	Peptidoglycan in the bacterial cell wall is _____	thin layer structure	tri crystal structure	cuboidal structure	crystal lattice structure	crystal lattice structure
21	Thickness of gram positive cell wall	10-20nm	20-80nm	80-100nm	10-50nm	20-80nm
22	Archae bacteria have	Pseudopeptidoglycan	murein	peptidoglycan	peptide	Pseudopeptidoglycan
23	Murein is a ____	protein	polymer	aminoacid	lipid	polymer
24	The Phosphatidic acid intermediate of phospholipids synthesis is activated by _____.	Cytosine triphosphate	Pyruvic acid	Flavoprotein	Malonyl CoA	Cytosine triphosphate
25	The process of nitrogen fixation requires energy from___.	ADP	AMP	ATP	FAD	ATP
26	The amino acid_____can be formed from the reaction of ammonium ions with alpha keto glutarate	D-glutamate	L-Lysine	L-glutamate	Mesodipimelic acid	L-glutamate
27	The amino acid L- glutamate can be formed from the reaction of ammonium ions with alpha keto glutarate by a	Reductive amination	Phosphorylation	Fermentation	Fixation	Reductive amination

	pathway known as____.					
28	The ability to transform the amino group of one amino acid to form another amino acid by a process known as_____.	Fermentation	Trans amination	Phosphorylation	Reductive amination	Trans amination
29	_____serves as the nitrogen source for all the amino acids.	D-glutamate	L-glutamate	L-Lysine	Mesodipimelic acid	L-glutamate
30	L-glutamate can react with 3-phosphoglycerate an intermediate of the glycolytic pathway to form the amino acid ____.	D-glutamate	L-Lysine	L-glutamate	L-serine	L-serine
31	_____is a precurssor for the biosynthesis of the amino acid L-glycine and L- cystein	L-serine	Mesodipimelic acid	L-glutamate	L-Lysine	L-serine
32	_____is a sulfur containing amino acid.	L-Lysine	L- cystein	L-serine	L-glutamate	L- cystein
33	The transformation of L-serine to L-cystein involves a reaction with_____.	Hydrogen sulfide	Ammonia	Sulphuric acid	Methane	Hydrogen sulfide
34	The formation of the aromatic ring structure involving the intermediate metabolite____.	Shikimic acid	Formic acid	Sulphuric acid	Acetic acid	Shikimic acid
35	During the biosynthesis of pyrimidines_____is formed from aspartate and carbamoyl phosphate	Adenosine phospho sulfate	Uridine triphosphate	Ammonium phosphate	Cytidine triphosphate	Uridine triphosphate
36	_____is a nucleotide in DNA and RNA.	Uridine triphosphate	Adenosine phosphosulfate	Cytidine triphosphate	Ammonium phosphate	Cytidine triphosphate
37	Carbon dioxide and methyl group donated from_____are also essential for the formation of the purine ring skeleton	Formic acid	Folic acid	Butyric acid	Cytidic acid	Folic acid
38	Biosynthesis of the adenine ring involves the substitution of an _____group for a keto group.	Acidic	Keto	amino	alpha	amino

39	_____metabolic steps are involved in the formation of the basic purine ring structure.	10	3	5	8	10
40	_____is the most important of the transaminase enzyme.	Epimerase	Cytidine triphosphate	Glutamate transaminase	Adenosine phosphosulfate	Glutamate transaminase
41	The biosynthesis of peptidoglycan is essential for cell growth and__of bacteria.	Division	Reproduction	Mating	Survival	Division
42	Peptidoglycan is a polysaccharide composed of N- acetylglucosamine and _____.	Murine layer	Mesodipimelic acid	L-glutamate	N-acetylmuramic acid	N-acetylmuramic acid
42	The enzyme that adds PEP to N-acetylglucosamine UDP is inhibited by _____antibiotic.	Bacitracin	Phosphonomycin	Streptomycin	Dapson	Phosphonomycin
43	The assembly of precurssors of peptidoglycan during cell wall synthesis takes place in the _____.	cytoplasm	Ribosome	Nucleus	Chloroplast	cytoplasm
44	The pyrophosphate is specifically inhibited by the_____antibiotic.	Streptomycin	Phosphonomycin	Bacitracin	Vancomycin	Bacitracin
45	The translocation step of peptidoglycan synthesis is inhibited by the antibiotic _____.	Bacitracin	Vancomycin	Phosphonomycin	Streptomycin	Vancomycin
46	Several enzymes involved in peptidoglycan synthesis bind to penicillin are called____.	Penicillinase	Precursor of penicillin	Bactoprenol	Penicillin-binding protein	Penicillin-binding protein
47	In lipopolysaccharide biosynthesis _____serves as the lipid carrier.	Bactoprenol	UTP	PAPS	Adenosine phosphosulfate	Bactoprenol
48	The lipid A layer of lipopolysaccharide is assembled in the_____.	Nucleus	cytoplasmic membrane	Chloroplast	Ribosome	cytoplasmic membrane
49	The fatty acid molecule seen in lipopolysaccharide is __.	Folic acid	Formic acid	Beta-hydroxymyristic	Phosphoric acid	Beta-hydroxymyristic

				acid		acid
50	The transfer of LPS molecules to the outer layer is done through___.	Adhesion sites	Peptidoglycan	Exosporium	Protoplast	Adhesion sites
51	Extracellur proteins that aid in the establishment and maintainance of disease are called_____.	Viral proteins	Virulence factors	Alpha toxin	Fibrins	Virulence factors
52	The enzyme that uses the phospholipid lecithin as the substrate is called_____.	Fructolipases	Phospholipids	Lecithinases	Leukocidins	Lecithinases
53	_____are the lytic agents capable of lysing white blood cells and may decrease host resistance	Leukocidins	Fructolipases	Lecithinases	Phospholipids	Leukocidins
54	_____serves as lipid carrier in the synthesis of peptidoglycon	N acetyl glucose amine	Vactoprenol	Galactose	Ethanolamine	Vactoprenol
55	_____connects the cytoplasmic membrane and outer membrane in gram –ve bacteria	LPS molecule	Beyer junction	Biotin	Hydroxyl butarate	Beyer junction
56	The APS is phosphorylated by a second ATP molecule to form _.	PAPS	ATP	ADP	AMP	PAPS
57	Carbon source for autotrophs	organic compounds	inorganic compounds	C	CO2	CO2
58	The reduced secondary quinine transfers its electrons to_____.	Cytochrome a	Cytochrome Bc1 complex	Cytochrome b	Both b and c	Cytochrome Bc1 complex
59	Reaction center bacteria chlorophyll absorb maximally at __.	810nm	800nm	840nm	820nm	840nm

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

PHOTOSYNTHESIS

Photosynthetic bacteria

There are three groups of photosynthetic bacteria: the purple bacteria, the green bacteria, and the cyanobacteria. The cyanobacteria differ most fundamentally from the green and purple photosynthetic bacteria in being able to carry out oxygenic photosynthesis. They use water as an electron donor and generate oxygen during photosynthesis. In contrast, purple and green bacteria use anoxygenic photosynthesis. Because they are unable to use water as an electron source, they employ reduced molecules such as hydrogen sulfide, sulfur, hydrogen, and organic matter as their electron source for the generation of NADH and NADPH. Consequently, purple and green bacteria do not produce oxygen but many form sulfur granules. Purple sulfur bacteria accumulate granules within their cells, whereas green sulfur bacteria deposit the sulfur granules outside their cells. The purple nonsulfur bacteria use organic molecules as an electron source. There also are differences in photosynthetic pigments, the organization of photosynthetic membranes, nutritional requirements, and oxygen relationships.

Normally green and purple bacteria are anaerobic and use H_2S and other reduced electron donors during photosynthesis. Because these bacteria grow best in deeper anaerobic zones of aquatic habitats, they cannot effectively use parts of the visible spectrum normally employed by photosynthetic organisms. There often is a dense surface layer of cyanobacteria and algae in lakes and ponds that absorbs a large amount of blue and red light. The bacteriochlorophyll pigments of purple and green bacteria absorb longer wavelength, far-red light not used by other photosynthesizers. In addition, the bacteriochlorophyll absorption peaks at about 350 to 550 nm enable them to grow at greater depths because shorter wavelength light can penetrate water farther. As a result, when the water is sufficiently clear, a layer of green and purple bacteria develops in the anaerobic, hydrogen sulfide-rich zone.

1. Cyclic Photophosphorylation:

When the photosystem I antenna chlorophylls funnel light energy to the reaction centre chlorophyll P700, the latter gets excited and, as a result, its reduction potential becomes very negative. The excited or high-energy electron of P700 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 b563 → plastaquinone → cytochrome b6 → cytochrome *f* → plastocyanin) back to oxidized P700.

Since the electrons travel in a cyclic pathway (i.e. they originate from P700 and come back to the P700),

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 firat step in oxygenic electron flow, the splitting of water (photolysis) into oxygen atoms (1/82 O_2) and hydrogen ions (2H). Photolysis donares an electron to the oxidized P_{680} molcule 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 arc 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^{2-}, S^0 and even Fe^{2+}) as externalelectron donors. When H_2S is the electron donor, globules of sulphur (S^0) are stored inside the cells of 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 P840 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.

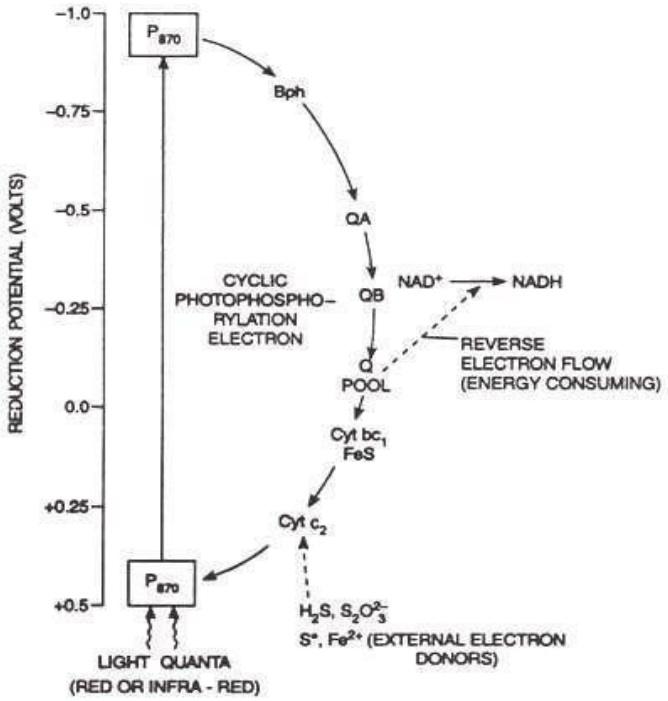


FIG. 25.5. Scheme of electron flow (cyclic photophosphorylation) in anoxygenic photosynthesis in purple bacteria.

Nitrogen metabolism

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 (NO_2) by bacteria like Nitrosomonas, Nitrococcus, etc. and then to nitrate (NO_3) by Nitrobacterium. Bacteria involved in nitrification are called chemoautotrophs. Here is the reaction involved in the process of nitrification.

$$2\,NH_3 + 3O_2 \longrightarrow 2NO^- + 2H^+ + 2H_2O$$

$$2$$

$$2NO_2^- + O_2 \longrightarrow 2NO_3$$

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

S.no	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Phototrophic anoxygenic bacteria utilize_____to generate NADH.	H2S	H2SO4	HNO3	NaCl	H2S
2	Bacteria that utilize malate as electron donor is said to be __.	Photoorganotrophic	Photo autotrophic	Photo heterotrophic	Photolithotrophic	Photoorganotrophic
3	aids in the motility of gliding bacteria.	Slime	Fimbriae	Flagella	Pili	Slime
4	Sporulation takes place for 10 hrs in	Streptococcus	Bacillus megaterium	Bacillus anthraces	Corynebacterium	Bacillus megaterium
5	The synthesis of flagella involves genes	20-30	40-50	15-30	30-40	20-30
6	The information required for flagella construction is present in the structure of .	Flagellin	hook	filament	basal body	Flagellin
7	Bacterial flagella anchor in to the cell wall and membrane by means of the ----------	Pilin	Stalk	Periplasm	Basal body	Basal body
8	---------- are membrane bound organelles in eukaryotic cells that contain very powerful digestive enzymes.	Mesosomes	Lysosomes	Metasomes	Trisomes	Lysosomes
9	Mycoplasma is an example of ---- bacteria	Gram negative	Cellwall high	Neutral	Cellwall less	Cellwall less
10	Flagella of Spirocheates are called flagella	Triplasmic	Periplasmic	Metaplasmic	megaplasmic	Metaplasmic
11	The peptidoglycan materials found in archae bacterial cell wall is called	Glycopeptide	Mucopeptine	Pseudomurein	Muramic acid	Pseudomurein
12	_____is not a organic compound	fixed carbon	reduced carbon	organic carbon	aminoacid	aminoacid

13	Phototrophy is the process by which organisms trap	chemicals	light energy	inorganic compounds	organic compounds	light energy
14	Cyanobacteria get their colour from the bluish pigment	chlorophyll b	chlorophyll a	phycocyanin	xanthophyll	phycocyanin
15	_____highly visible blooms that can form in both freshwater and marine environments.	green sulphur bacteria	cyanobacteria	photosynthetic bacteria	fungi	cyanobacteria
16	Photopigments associated with purple and green bacteria are	Bacteriochlorophyll	Bacteriophytin	Cyanobacteria	Rhophytin	Rhophytin
17	Anoxygenic photosynthesis is carried out by _____	Photosynthetic bacteria	Cyanobacteria	Algae	Fungi	Cyanobacteria
18	Photosynthetic apparatus present in Cyanobacteria are __	Chloroplasts	Thylalkoids	Chlorophylls	Cytoplasm	Thylalkoids
19	_____organism is involved in the production of dextran from sucrose.	*Bacillus subtilis*	*Pseudomonas aerogenosa*	. *Streptococcus mutans*	*Proteus*	. *Streptococcus mutans*
20	Bioluminescence involves the oxidation of a Luciferin in the presence of ____ enzyme	Luciferase	Protease	Cellulase	Ligase	Luciferase
21	_____is light produced by a chemical reaction c in an organism.	Bioremediation	Biodetoriation	Bio degradation	Bioluminescence.	Bioluminescence
22	_____is the commonest cause of luminescence in the surface water of seas.	Dinoflagellates	Ctenophores	Cephalopods	Urochordates.	Dinoflagellates

23	_____is a large protein which an make up 2-5-% of soluble protein in luminescent bacteria	Cellulase	Ligase	Luciferase	Protease	Luciferase
24	In photolithotrophic microbes ATP is used in synthesis of cell constituent from __	Carbonmonoxide	$C0_2$	O_2	N_2	N2
25	BGA the source of H_2 is H_20 which is thereby oxidized to	Molecular 0_2	$C0_2$	O_2	N_2	Molecular 0_2
26	An example of direct photoassimilation of an organic substrate is _____	*Rhodospirilum rubrum*	*Rhodospirilum prostratum*	Both	None	*Rhodospirilum prostratum*
27	In the dark, some of the photoorganotrophic bacteria oxidise organic substrate through	TCA cycle	Glycolysis	EMP pathway	Oxidation	TCA cycle
28	In photosystem I & II conversion of light energy in to _ energy occurs.	Thermal	Chemical	Physical	Biological	Chemical
29	The reactive pigment in photosystem I is __	Dual e^- carrier	Single e^- carrier	Protons carrier	Neutron carrier	Single e^- carrier
30	The pigment used in photosystem I is __	P_{700}	P_{500}	P_{400}	P_{600}	P_{700}
31	The standard reduction potential of the reaction centre in photosystem is _	450 mv	550 mv	650 mv	750mv	450 mv
32	Electrons expelled from photosystem I are accepted by _____molecules.	Ferridoxin	Ferrodoxin	Ferricdoxin	Ferrousdoxin	Ferrodoxin
33	Example for prokaryotic photosynthetic organism	Cyanobacteria	Red algae	Higher plants	Lower plants	Cyanobacteria

34	Photosynthesis means ___	Light energy to chemical energy	Chemical energy to light energy	Light energy to physical energy	Physical energy to light energy	Light energy to chemical energy
35	Example for green non sulfur bacteria	Chloroflexaceae	Chlorobiaceae	Chromatiaceae	Thiorodaceae	Chlorosomes
36	Example for green sulfur bacteria	Chloribiaceae	Chloroflexaceae	Thiorodaceae	Chlorobiaceae	Chlorobiaceae
37	Purple sulfur bacteria	Chloribiaceae	Chromatiaceae	Thiorodaceae	Chloroflexaceae	
38	Bacteriochlorophyll are located in ___	Chlorosomes	Mesosomes	Metasomes		
39	Other name for Thiorhodaceae	Purple sulfur bacteria	Green sulfur bacteria	Green non sulfur bacteria	Green algae	Purple sulfur bacteria
40	Thiospirillum occurs in __shape	Kidney	Heart	Liver	Round	Kidney Rhodospirillum
41	Non-motile form of Rhodospirillacaea is	Rhodocyclus	Rhodospirillum	Rhodococcus	Azospirillium	Rhodospirillum
42	Organic compound is utilized by ___	Green non sulfur bacteria	Purple sulfur bacteria	Green sulfur bacteria	Photosynthetic bacteria	Green sulfur bacteria
42	H_2S is utilized by	Green sulfur bacteria	Purple sulfur bacteria	Green sulfur bacteria	Green non sulfur bacteria	Purple sulfur bacteria
43	H_2S is utilized by	Green sulfur bacteria	Purple sulfur bacteria	Green sulfur bacteria	Green non sulfur bacteria	Purple sulfur bacteria
44	Example for oxygenic photosynthesis	Cyanobcateria	Blue green algae	Red algae	Green algae	Green algae
45	Gas vacuoles are needed for	Metabolism	Buoyancy	Catabolism	Transport	Buoyancy
46	Green sulfur bacteria exist in _____rich zone lakes	Sulfur	Iron	Copper	Nickel	Iron
47	The Oxidation of ethanol was strictly_____ dependent	H_2	O_2	N_2	Co_2	Co_2

48	_____using sulfate as terminal e acceptor	*Desulfovibrio*	*Methano bacterium*	*Nitrobacillus*	*Hydrogenomo na s*	*Desulfovibrio*
49	Enzyme catalyzing sulfate to adenosine 5 – phosphosulfate is	Pyrophosphat ase	Sulfate adenylyl transferase	Adenyly sulfate kinase	Sulfite reductase	Pyrophosphatase
50	The process of conversion of light energy from the sun to chemical energy with in ATP is known as__.	Photophospho rylation	Substrate level phosphorylation	Oxidative phosphorylatio n	Chemiosmos is	Photophosphorylat ion
51	The membrane bound carriers are collectively known as _____	Oxidation reduction potential	Photosystem	Chemiosmosis	Phosphoryla tion	Photosystem
52	The light is captured by light harvesting__ pigments.	Antenna	Flagella	Pili	Fimbriae	Antenna
53	An example for anoxygenic photoautotrophic bacteria is _____.	Cyanobacteria	Green bacteria	Purple sulphur bacteria	None	Purple sulphur bacteria
54	_____synthesis chlorophyll b in addition to chlorophyll a.	Cyanobacteria	Anabaena	Nostoc	algae	Cyanobacteria
55	The vesicles produced by green photoautotrophic bacteria are known as___.	Chlorosome	Vacuole	Centromere	Both a and b	Chlorosome
56	_____use organic acid as electron donors.	*Pseudomonas sp*.	*Bacillus sp*.	*Rhodococcus sp*.	None	*Rhodococcus sp*.
57	When cyanobacteria utilize _____they form elemental sulphur granules.	HCl	H2S	H2SO4	All the above	H2S
58	Photosystem I is otherwise known as_____.	Z pathway	Non-cyclic oxidative phosphorylation	Cyclic oxidative phosphorylati o n	Photosystem I & II	Cyclic oxidative phosphorylation
59	How many protons are picked during the passage of electrons	8	12	16	4	4

	through the carriers of photosystem I.					
60	The protons used to reduce an oxidized carrier are known as _____.	Seconda ry quinine carrier	Primary quinine carrier	Tertiary quinine carrier	All the above	Secondary quinine carrier

Reg. No. : ----------

[19MBU202]

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
FIRST INTERNAL ASSESSMENT, DECEMBER - 2019
SECOND SEMESTER
MICROBIOLOGY
MICROBIAL PHYSIOLOGY AND METABOLISM

Time: 2 hours **Maximum: 50 marks**
Date: 19/12/2019 [AN] **Class: I B.Sc. MB**

PART A – (20 x 1 = 20 marks)
Answer all the questions

1. Chemolitho heterotrophs are also called as _____
   a) Mixotroph b) Auxotroph
   c) Chemotroph d) Lithotroph
2. Vitamin B6 is otherwise called as _____
   a) Riboflavin b) Cyanocobalimine
   c) Pyridoxine d) Ribtol
3. Microorganism use _____to uptake nutrients.
   a) ferridoxin b) chlorophyll
   c) siderophores d) light
4. Bacterial species which grows as phototroph under anaerobic condition and as chemotroph under aerobic condition is _____
   a) *Rhodospirillum rubrum* b) *Rhodospirillum stratum*
   c) *Proteus* d) *Vibrio*
5. Shape of bacterial growth curve is _____
   a) Straight b) Curved
   c) Sigmoid d) Round
6. Synchronous culture _____
   a) Further no growth b) cell divides slowly
   c) All cell divide simultaneously d) fast cell growth
7. Petroff Hausser counting chamber _____
   a) Measuring the cell b) view the motility
   c) View the shape d) measure the size
8. Microbial cultures composed of cells that are all the same stage of the cell cycle are called
   a) Auxenic culture b) Mixed culture
   c) Pure culture d) Synchronous culture
9. The time required for the doubling of cell mass is known as _____
   a) Doubling time b) Generation time
   c) Generation gap d) Developing time
10. The growth is modified by controlling and monitoring the turbidity of the culture is called _____
    a) Synchronous method b) Batch culture method
    c) Turbidostat d) Thermostat
11. Cyanobacteria are _____
    a) Chemoautotrophs b) Lithotrophs
    c) Photoautotrophs d) Chemotrophs
12. Nitrogen is required for the production of what category of molecules_____
    a) Fatty acid b) phospholipids
    c) Nucleotide d) Carbohyrates
13. Reproduction of bacterial cells take place by _____
    a) Pollution b) Binary Fission
    c) Mitosis d) Meisos
14. Doubling time is otherwise called as_____.
    a) Growth curve b) Growth rate
    c) Generation time d) Generation period
15. The log growth phase is also called as _____.
    a) Stationary phase b) Exponential growth phase
    c) Lag phase d) Death phase
16. The peptidoglycon layer of Gram negative bacteria is located in the ---------- space
    a) Triplasmic b) Metaplasmic
    c) Periplasmic d) Epiplasmic
17. Microorganisms pathogenic for humans and other warm blooded animals grow best at a temperature of _____
    a) 40^{o}C b) 37^{o}C
    c) 35^{o}C d) 20^{o}C
18. Endospores can resist _____
    a) Low salinity b) salinity
    c) Water activity d) Desiccation
19. Nitrogen is an essential element of the _____ that make up protein
    a) Amines b) Amino acids
    c) Hydroxyl d) Carboxyl
20. Organism that make use of carbon dioxide as their main source of carbon are _____
    a) Autotrophy b) Heteotroph
    c) Chemotroph d) Lithotroph

PART B – (03 x 02 = 06 marks)
Answer all Questions (All questions carry equal marks)

21. What is meant by Population doubling time?
22. Define the terms Thermophiles and Psychrophiles.
23. Define the terms Acidophiles and Alkaliphiles.

PART C – (03 x 08 = 24 marks)
Answer all questions choosing either a (or) b. (All questions carry equal marks)

24. Explain in detail about the Macronutrients and their Physiological functions.
    (OR)
    Write a brief note on passive diffusion and facilitated diffusion.
25. Give a detail account on viable count and turbidity method.
    (OR)
    Give a brief account on group translocation and iron uptake.
26. Explain in detail about the Micronutrients and Growth factor with their Physiological functions.
    (OR)
    Write in detail about different phases of microbial growth with proper example