Agricultural Microbiology

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The future lies in agriculture, those who understand the land will change the world.

Course Name	Agricultural Microbiology
Lesson 1	History of Microbiology: Evolution of Biogenesis
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Learning Objectives

1. Understanding the significant contribution made by stalwarts in microbiology.
2. To receive a basic knowledge of the mother principles of microbiology on which the current understanding stands.

1. Introduction

- With the discovery of living forms which were invisible to our eyes led to a milestone in the history of microbiology and it was even more before in the 13th century people started believing that "invisible" entities were responsible for diseases.
- During the last quarter of the 19th century, the word "*microbe"* was coined to describe these organisms, all of which were thought to be related.

2. Definition of Microbiology

- It is the study of microbes and includes a wide group of generally minute simple life forms, which includes bacteria, fungi, protozoa, viruses etc.
- Microbiology deals with the structure, function, and classification of microbial groups.

3. Important events in the field of Microbiology

- Antony Van Leeuwenhoek's lucid report on the ubiquity of microbes enabled Louis Pasteur 200 years later to discover the involvement of these creatures in fermentation reactions and allowed Robert Koch to discover the association of microbes with disease.
- Koch was remembered for his isolation of the bacteria that causes anthrax and tuberculosis. (Koch received the Nobel prize in 1905).

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- Microscope- Microbiology began when people learned to grind lenses from piece of glass and combine them to produce magnification great enough to enable microbes to be seen.
- Thirteen century-Roger Bacon postulated that disease is produced by invisible living creatures. These types of suggestion were given by other scientist [Antony Van Plenciz-1762; Fran Castro].
- Athanasius- referred to “worms” invisible to naked eye in decaying bodies, meat, milk etc. Although his description lacked accuracy, Kircher was the first person to recognize the significance of bacteria and other microscope in disease.
- 1665- Robert Hooke’s description of cells in a piece of cork established the fact that bodies of animals and plants, complex as they may appear, are yet composed of a few elementary parts frequently repeated.
- Although he was probably not the first to see bacteria and protozoa, Antony Van Leeuwenhoek was the first to report his observation with accurate description and drawing. Leeuwenhoek carefully recorded his observations in a series of letters to British Royal Society. In one of the first letter, he mentioned the “very little animalcules” which we recognise as free-living protozoa.

Table 1. Contributions of key scientists in Microbiology

Era	Key Scientist	Contribution
1600-1700	F. Redi	Performed experiment to disprove spontaneous generation
	Leeuwenhoek	Accurately record and report micro-organisms
	Jenner	Vaccination for smallpox
1800-1900	T. Schwann and F. Schultze	Disprove spontaneous generation
	Justus von Liebig	Physicochemical theory of fermentation
	Jacob Henle	Principle of germ theory of disease

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	Oliver Holmes	Contagiousness of Puerperal fever
	Louis Pasteur	Established Germ theory of fermentation Germ theory of Disease Discovered protozoa causing pebrine disease in silk worm Demonstrated virulence
	Joseph Lister	Developed aseptic techniques
	John Tyndall	Tyndallisation / fractional sterilization concept
	F. Hesse	Use of agar in microbiology
	Robert Koch	Koch Postulates and Pure culture techniques
	H. Christian Gram	Differential staining (Gram staining)
	Serge Winogradsky	Discovered N fixing bacteria in soils Nitrification Discovered anaerobic nitrogen fixing bacterium *Clostridium pasteurianum*
1900-1910	Bordet and Gengou	Complement fixation reaction
	MartinusBeijerink	Principle of enrichment culture, finding of first virus, isolated sulphur reducing bacteria,
	Twort and Herelle	Independently discovered bacteriophage

4. Spontaneous generation vs. biogenesis: Related thoughts and experiments

- The early Greeks believed that living things could originate from non-living matter (abiogenesis) and that the goddess Gea could create life from stones.
- Aristotle was of the view that animals might originate spontaneously from the plants, soil or other unlike animals (considered as spontaneous generation). It was accepted that maggots could be produced spontaneously by exposing meat to warmth and air.
- Francesco Redi doubted this and conducted one experiment in which he placed a meat in a jar covered with gauze. Attracted by the odour

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of the meat, flies eggs on the covering, and from the eggs maggots developed. Hence, the experiment established the facts that the origin of the maggots was the flies and not the meat.

- 1749- John Needham while experimenting with meat exposed to hot ashes, observed the appearance of organisms not present at the start of the experiment and concluded that bacteria originated from the meat.
- At the same time, Lazaro Spallanzani boiled beef broth for an hour and then sealed the flasks. No microbes appeared following incubation, but his results confirmed in repeated experiments, failed to convince Needham, who insisted that air was essential to the spontaneous production of microscopic beings and that it had been excluded from the flask by sealing them.
- This argument was answered some 60 or 70 years later independently by two other investigators, Franz Schulze and Theodor Schwann. Schulze passed air through strong acid solution into boiled infusions; whereas Schwann passed air into his flasks through red hot tubes. In neither case did microbes appear. But the diehard advocates of spontaneous generation were still not convinced. Acid and heat altered air, so that it would not support the growth, they said.

- 1850-H.Schroder and T.VonDusch performed a more convincing experiment by passing air through cotton flask containing heated broth. Thus the microbes were filtered out of air by the cotton fibres so that growth did not occur; a basic technique of plugging bacterial culture tubes with cotton stoppers was initiated.

- The concept of spontaneous generation was revived for the last time by F.A. Pouchet, who in 1859 produced an extensive report proving the occurrence of spontaneous theory.

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- Irritated by Pouchet's logic and data, Pasteur performed experiments that ended the argument for all time. Louis Pasteur designed several bottles with S-curved necks (Goose neck opening). A nutrient enriched broth was added in one of the bottles and it was boiled. Life was not seen in the jar for a year. Then the bottle was tilted exposing the inner content more directly to the air in the swan neck and life-forms were seen in the broth in few days. It was concluded that as long as dust and other airborne particles were trapped in the S-shaped neck of the bottle, no life was created. But on tilting the bottle the medium came in contact with the air trapped in the S- shape of the bottle's opening and life forms grew. This experiment by Louis Pasteur disapproved the Spontaneous generation theory, which stated that life was generated from inanimate matter.

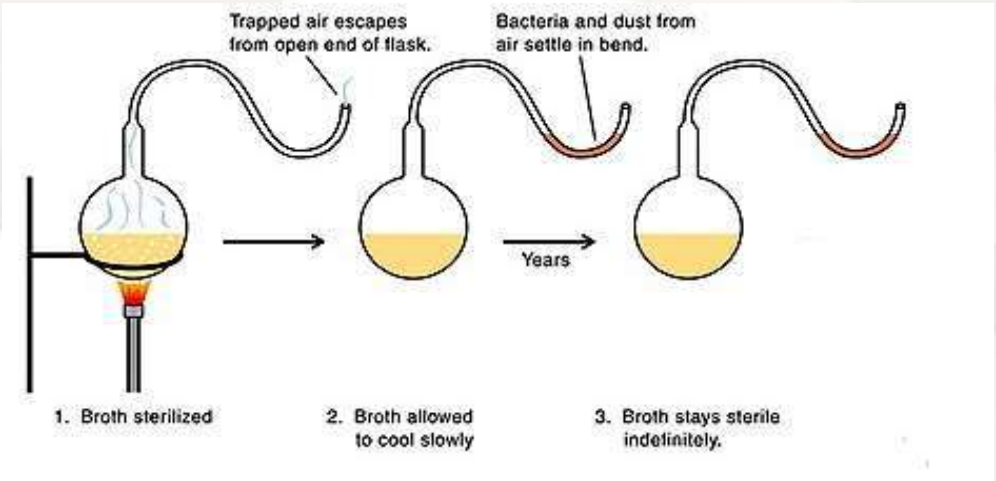

Figure 1. The theory of spontaneous generation was disproved with the devices illustrated above. (https://www.toppr.com/ask/question/which-of-the-following-theory-was-disapproved-by-swan-neck-experiment-of-louispasteur/)

- Finally, John Tyndall conducted experiments in a specially designed box to prove that dust carried the germs. He demonstrated that if no

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dust was present, sterile broth remained free of microbial growth for indefinite periods.

5. References

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Course Name	Agricultural Microbiology
Lesson 2	Germ Theory of Diseases and Immunity Concepts
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Learning Objectives

1. Understanding the role of microbe behinds every disease they cause.

2. To develop an idea behind the development of vaccines using immunization theory.

1. Germ theory of disease: Definition and related events

Germ theory of disease states that pathogen / microbes / germs are responsible for causing diseases. These tiny organisms used to invade the living host and grow within the host causing related diseases. Germ not only refers to bacteria but a wide range of microorganisms ranging from protest to viroid's. Even from 1546, people started working and expanding the knowledge about the ability of germs to cause diseases. A series of events are placed here for understanding:

- Fran Castro suggested that diseases might be due to invisible organisms transmitted from one person to another.
- Von Plenciz (1762) described that living agents are the cause of disease. Different germs are responsible for different diseases in 1836.
- Robert Koch (1876) concluded the germ theory of disease by working on anthrax disease on animals (sheep). His experiments and observations led to the establishment of Koch's postulates, which provided the guidelines to identify the causative agent of infectious diseases.
- Oliver Holmes insisted that puerperal fever, a disease of childbirth caused by germ carried from one mother to another midwives and physicians (Wrote Contagiousness of Puerperal Fever).

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- I.P. Semmelweis initiated the use of antiseptics in obstetrical practice, which minimised the chances of infection.
- Joseph Lister shown the importance of antisepsis in medical field.
- Louis Pasteur following the germ theory identified and isolated the parasite causing pebrine (a silkworm disease).
- Pasteur then tackled the problem of anthrax.

2. Koch's Postulates

Robert Koch in Germany was working on anthrax and isolated bacilli responsible for the death of cattle's due to anthrax. He isolated and injected the isolated microbes into other animals to see the symptoms of anthrax. Eventually, with his experiment he established the postulates presented hereunder:

1. A specific organisms can be found in association with a given disease.

2. The organisms can be isolated and grown in pure culture in the laboratory.

3. The pure culture will produce the disease when inoculated into a susceptible animal.

4. It is possible to recover the organism in pure culture from the experimentally infected animal.

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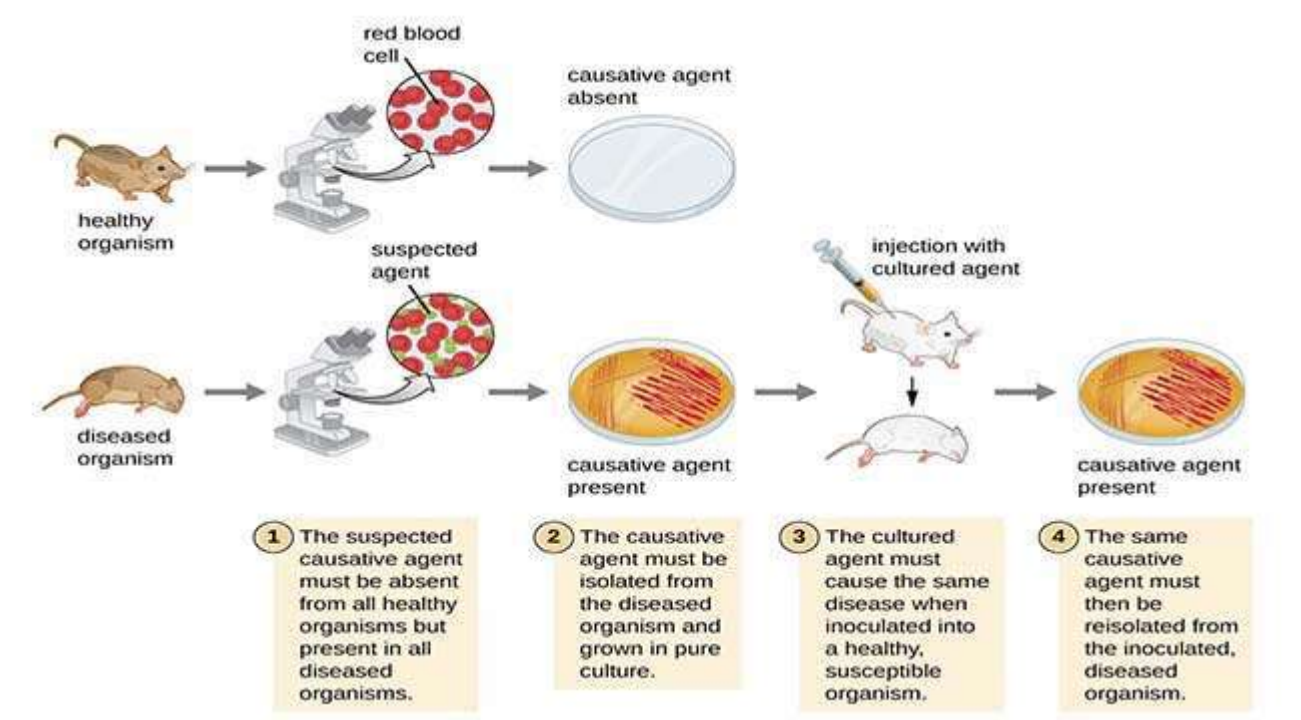

Figure 1. Koch's Postulates explained through the above illustration (https://microbenotes.com/kochs-postulates-and-its-limitations/)

3. Laboratory techniques and pure culture

- Microbes are ubiquitous in nature and are present in extremely large population made up of many species. If someone wants to study a particular species, it needs to be separated with due care.
- Established lab procedures are there to isolate specific microorganisms representing each species and to grow each species separately. The growth of a mass of cells of the same species in a laboratory vessel (such as test tube) is called pure culture.

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- The concept of pure culture was first proposed by Joseph Lister in the year 1878 using serial dilutions technique. Using this technique, one can isolate microorganisms responsible for specified cause.
- Koch was knowns as one of the best bacteriologist that time was refining methods for the study of bacteria. He used agar and added gelatine and other solidifying material such as agar to media in order to obtain isolated growth of microorganisms known as colonies, each of which contained millions of individual bacterial cells packed tightly together. From these colonies, pure culture could be transferred to other media.
- With this technique, Koch was able to isolate the microorganism responsible for tuberculosis.

4. Protection against infection

- Edward Jenner in 1798 used Cowpox Virus to immunize people from small pox. But the science behind this technique was not known at that time.
- Pasteur isolated the bacterium responsible for chicken cholera and grew it in pure culture. He has taken two batches of chickens and he inoculated one batch with attenuated cultures (cultures of several weeks old) and the other batch with virulent (fresh culture) cultures. The batch that was inoculated with attenuated cultures developed resistance and the other batch died. So, in some way bacteria could lose their ability to produce disease i.e. their virulence after standing for long time. But, these attenuated culture retained their capacity for stimulating the host to produce antibodies. That protect against subsequent exposure to virulent organisms.
- With this experiment, the concept of Jenner's successful use of cowpox virus gets clear.

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- Pasteur applied the same concept for the prevention of anthrax and then termed these attenuated cultures as Vaccines. The term vaccines was derived from Latin vacca meaning cow, just to honour Jenner, otherwise attenuated culture had no connection with cows.

- In a service to humanity, Pasteur took the challenge and worked out to find a vaccine for hydrophobia. Finally, he succeeds in developing the remedy for rabies using the same principle.

Table 1. Diseases agents following principle of germ theory

Year	Disease of infection	Agent	Discoverer
1876	Anthrax	*Bacillus anthracis*	Koch
1880	Typhoid	*Salmonella typhi*	Eberth
1880	Malaria	*Plasmodium sp.*	Laveran
1882	Tuberculosis	*Mycobacterium tuberculosis*	Koch
1885	Tetanus	*Clostridium tetani*	Nicolaier
1894	Plague	*Yersinia pestis*	Kitasato and Yersin
1898	Dysentery	*Shigella dysenteriae*	Shiga
1905	Syphilis	*Treponema pallidum*	Schaudin and Hoffmann
1909	Rocky Mountain spotted fever	*Rickettsia rickettsii*	Rickets

With this advancement, new bacteria were being discovered frequently and their disease producing capacities were proven with Koch's postulates.

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Course Name	Agricultural Microbiology
Lesson 3	Bioenergetics and Respiratory Chain
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Learning Objectives

1. To develop a basic idea of free energy change of chemical reactions.
2. To understand the science behind electron transport chain.

1. Introduction

- The term metabolism denotes all the organized chemical activities performed by a cell.
- It comprises two general types: Energy production and Energy utilization.
- The study of bacterial metabolism focuses on the chemical diversity of substrate oxidations and dissimilation reactions (reactions by which substrate molecules are broken down), which normally function in bacteria to generate energy.
- Bacterial metabolism also includes the uptake and utilization of the inorganic or organic compounds required for growth and maintenance of a cellular steady state (assimilation reactions).
- These respective exergonic (energy-yielding) and endergonic (energy-requiring) reactions are catalysed within the living bacterial cell by integrated enzyme systems, the end result being self-replication of the cell.
- Hence, metabolism has an energy-generating component, called catabolism, and an energy consuming, biosynthetic component, called anabolism.
- Catabolism is the set of metabolic pathways that breaks down molecules into smaller units that are either oxidized to release energy or used in other anabolic reactions.
- Anabolism is the set of metabolic pathways that construct molecules from smaller units. These reactions require energy, known also as an endergonic process.

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2. Bioenergetics principles

- Most cells obtain energy by carrying out chemical reaction which liberates energy.
- In course of any chemical reaction, energy available for the performance of useful work is released or absorbed.
- The amount of energy liberated or taken up during the course of a reaction is referred as free energy change (ΔG).
- When ΔG of any reaction is negative, then there is a release of energy (Exergonic reaction).
- When ΔG of any reaction is positive, then the reaction requires energy (Endergonic reaction).
- ΔG^o is the standard free energy change (it means the change when 1 mole of reactant is converted to 1 mole product at 25ºC and 1 atm. pressure).
- $\Delta G^o = -RT \ln K_{eq}$(R-Gas constant; T- Absolute temperature and K_{eq}-Equilibrium constant).
- When ΔG^o is negative, the formation of product is favoured in a reaction.
- $\Delta G = \Delta G^o + 2.303\ RT \ln K_{eq}$

2.1. Common reactant phenomena in exergonic and endergonic reactions

- It is essential that energy released from exergonic reactions is to be used to drive endergonic reactions. This is only possible through a common reactant (coupling) phenomenon:
- Consider the two general reactions,

A ⟶ B (ΔG^o = -8000 cal) (Exergonic)……(1)

C ⟶ D (ΔG^o = +4000 cal) (Endergonic)……..(2)

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- The energy liberated by the first reaction is to be used to drive the second reaction by coupling the two reactions as:

$$A+X \longrightarrow B+Y \ (\Delta G^{o} = -3000cal) \ (Exergonic)\ldots\ldots\ldots\ldots\ldots(3)$$

$$C+Y \longrightarrow D+X \ (\Delta G^{o} = +1000\ cal) \ (Endergonic)\ldots\ldots\ldots\ldots..(4)$$

- In the reaction no. third, 5000 cal of original 8000 cal is used for the conversion of X to Y.
- In the reaction no. fourth, Y gets converted back to X, thereby releasing the 5000 cal to drive the conversion from 'C' to 'D'.
- Thus, the overall ΔG^{o} of the reaction no. 4 becomes +1000 cal (+4000 minus 5000 cal).
- The common reactant Y is referred to as an energy rich or energy transfer compound.
- The common reactants of greatest use to the cell are those capable of transferring large amount of free energy called high energy transfer compounds (e.g. ATP, GTP, UTP, Acetyl phosphate, PEP etc.)
- ATP is the energy currency ofcell in the exchange of energy between exergonic and endergonic reactions. Hydrolysis of ATP generates energy (ΔG^{o}= -7.3 kcal mol^{-1}). Oxidation-reduction is the commonest reaction for energy production.

2.2. Oxidation-reduction reactions in respiration

2.2.1. Concepts

- Oxidation – Loss of electrons (loss of hydrogen atoms).
- Reduction- Gain of electrons.
- Oxidizing agent- will absorb electrons and becomes reduced (e.g. Fe^{+3}, H^{+}, Fumaric acid).

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- Reducing agent- will donate electrons and becomes oxidised. (e.g. Fe^{+2}).
- In any chain reactions, a pair of substances is involved: one is reduced and the other as oxidized e.g. ferrous and ferric form. Each such pair of substances is referred as an oxidation-reduction system (O/R).
- One O/R system may tend to absorb electrons from another O/R system. The tendency to absorb electrons is measured by electromotive potential (E_o') of an O/R system.
- The more positive the E_o', the greater the oxidizing ability of the system.

2.2.2. Electron donor in respiration

- In respiration, an oxidizable substrate is the primary electron donor.
- In aerobic respiration, the terminal electron acceptor is oxygen.
- In anaerobic respiration, the final electron acceptor is a compound like fumarate, NO_3^-, SO_4^{2-}.
- In fermentation, an organic compound is the final electron acceptor.
- In photosynthesis by algae and cyanobacteria, water serves as a primary electron donor and $NADP^+$ as a terminal electron acceptor.
- The path through which these electrons flow in the various processes is called electron transport chains.

3. The Respiratory Chain

- When a pair of electrons from an oxidizable substrate is coupled with the reduction of an electron acceptor such as oxygen, there is a large free energy change.
- The flow of electrons through the transport chain allow a stepwise release of this energy, which is conserved in the form of ATP.
- A respiratory chain consists of enzymes having prosthetic group or co-enzymes. And co-enzymes can be regarded as an O/R system.

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3.1. Example of co-enzymes

- NAD (Nicotinamide Adenine Dinucleotide) and NADP (Nicotinamide Adenine Dinucleotide Phosphate)- Vitamin niacin is the precursor of both.
- FAD (Flavin Adenine Dinucleotide) and FMN (Flavin Mononucleotide)- Vitamin Riboflavin is in the structure of FAD and FMN. The reduced form of FAD and FMN are $FADH_2$ and $FMNH_2$ respectively.
- Co-enzyme Q- also called ubiquinone. It is a fat-soluble coenzyme. It functions as an acceptor of reducing power from the flavin linked dehydrogenase.
- Cytochromes- Three types are cytochromes a, cytochromes b and cytochromes c. The cytochromes act sequentially to transport electrons from coenzyme Q to O_2.

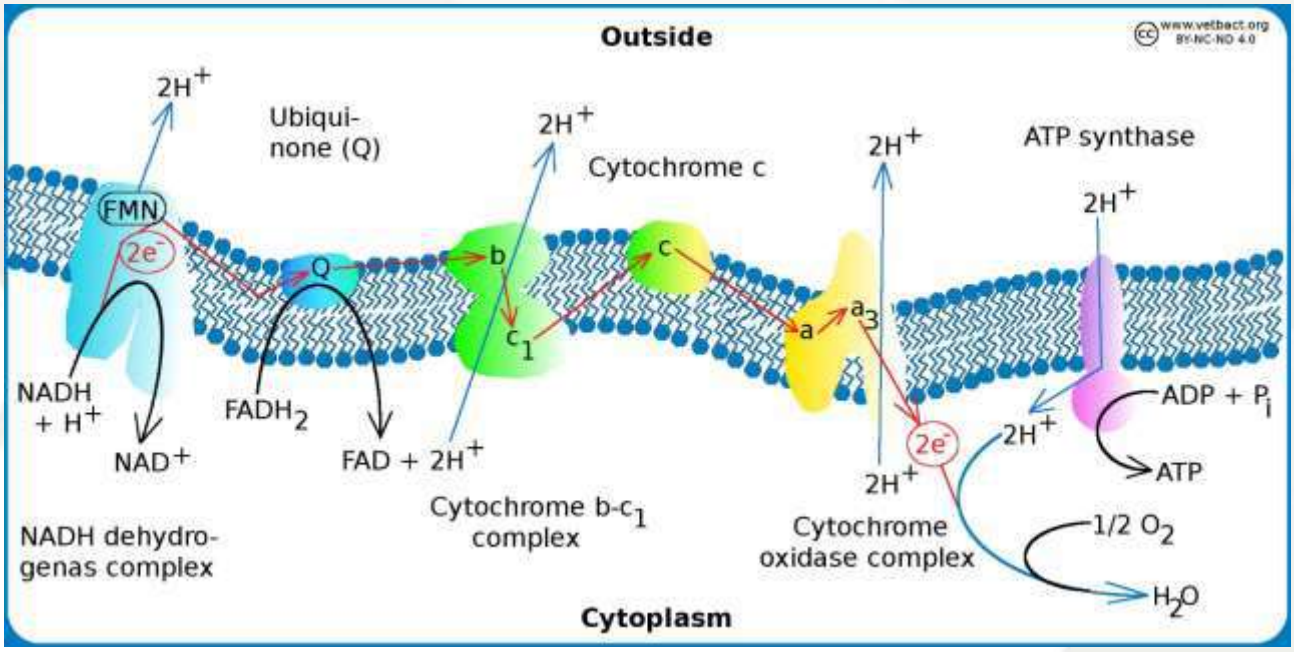

Figure 1. Schematic view of the electron transport chain in bacteria with aerobic metabolism. It shows a sequential oxidation steps where sufficient energy is liberated to permit synthesis of ATP. (https://www.vetbact.org/index.php?displayextinfo=127)

Course Name	Agricultural Microbiology
Lesson 5	Microbial Metabolism: Energy Production by Aerobic Processes
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Learning Objectives

1. To develop a basic idea of tricarboxylic acid cycle in respiration.
2. To understand the energy yield in aerobic respiration.

1. TCA Cycle (Citric acid cycle or Krebs cycle):

- The TCA cycle is the central metabolic pathway of the cell and is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or dicarboxylic acid.
- The tricarboxylic acid cycle (TCA cycle) is a sequence of reactions that generate energy in the form of ATP and reduced coenzyme molecules ($NADH_2$ and $FADH_2$).
- Many intermediates in the cycle are precursors in the biosynthesis of amino acids, purines, pyrimidines, etc. For example, oxaloacetic acid and α- ketoglutaric acid are amino acid precursors.
- Pyruvate is firstly carboxylated and converted into acetyl Co-A, which is the connecting link between glycolysis and TCA cycle and acts as a fuel for TCA cycle.
- Acetyl Co-A is a 2- carbon energy rich molecule which initiates TCA cycle and is condensed with a 4-carbon intermediate, oxaloacetate, to form citrate and to begin the 6- carbon stage.
- The citrate is isomerised to give isocitrate which is oxidised and decarboxylated twice to produce α- keto glutarate, then succinyl Co-A.
- During this, 2 NADH molecules are generated and 2 carbons are released from the cycle as CO_2.
- Succinyl Co-A is converted into oxaloacetate via formation of succinate, fumarate and L- malate.
- During 2 oxidation steps i.e., succinate to fumarate and 1- malate to oxaloacetate, $1FADH_2$& 1NADH produced. GTP is produced during conversion of succinyl Co-A to succinate.
- Finally, the oxaloacetate is reformed and becomes ready to join acetyl Co-A to proceed further.

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- Krebs cycle generates 2 CO_2 molecules, 3 NADH, 1 $FADH_2$ and 1 GTP molecules, for each acetyl Co-A molecule oxidized.

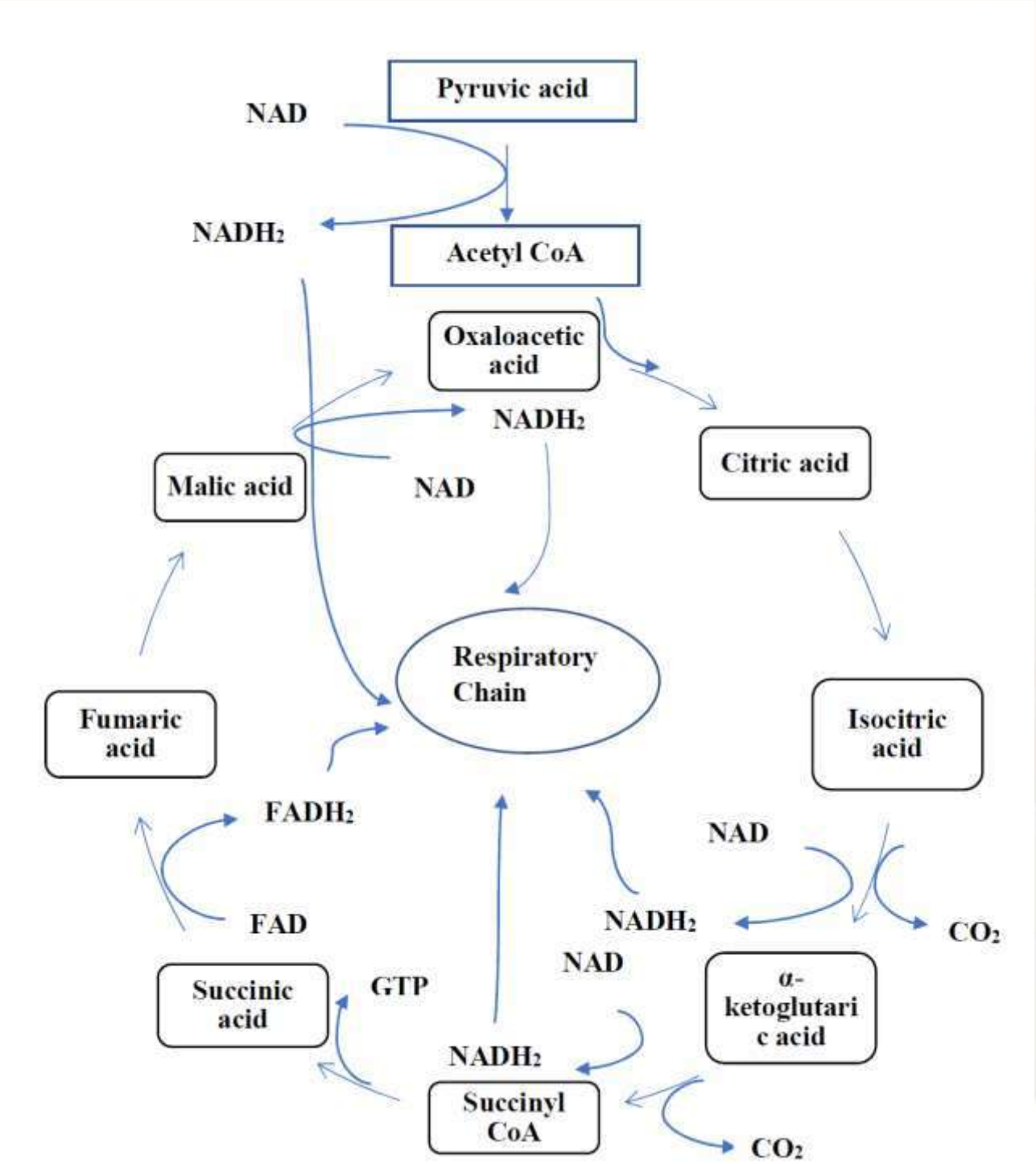

Figure 1. The TCA cycle

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- The generation of ATP from NADH and $FADH_2$ molecules associated with electron transport chain and oxidative phosphorylation.
- The generation of GTP molecule takes place via substrate level phosphorylation.
- The TCA cycle is an amphibolic cycle, which means that it functions not only in catabolic but also in anabolic (synthesis) reactions.

- Overall, reaction of the TCA cycle is

$$\text{Acetyl-CoA} + 3H_2O + 3NAD + FAD + ADP + Pi \rightarrow 2CO_2 + CoA + 3NADH_2 + FADH_2 + ATP$$

2. Energy yield in aerobic respiration

- For each glucose molecule broken down, there are 12 reduced coenzymes to be oxidized.
- 2 $FADH_2$ (I from each turn of TCA cycle) and 10 $NADH_2$ (2 from glycolysis; 2 from the gateway step between glycolysis and the TCA cycle and 6 from two turns of the TCA cycle).
- Since 3 ATP are produced from each NADH2 and 2 ATP from each $FADH_2$, there are 34 ATP generated from the reduced coenzymes via oxidative phosphorylation.
- The total yield of ATP from the aerobic respiration is 38 (34 from the oxidation of coenzymes, 2 from glycolysis and 2 from the side reaction of the TCA cycle).

3. Catabolism of Lipids

- Some organisms use lipid as an alternate source of energy.
- Lipids are converted into intermediate of glycolytic and TCA pathways.

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- Breakdown of lipids begins with the cleavage of triglycerides by the addition of water to form glycerol and fatty acids (Lipase enzyme).
- Glycerol can be converted to dihydroxyacetone phosphate which is broken down further.
- Fatty acids are oxidized by the successive removal of 2-carbon fragments in the form of acetyl CoA, known as β-oxidation.
- The acetyl CoA then enters the TCA cycle.

4. Catabolism of Proteins

- Bacteria secretes proteases to hydrolyse proteins to peptides.
- Peptidase that breaks down peptides to individual amino acids.
- When amino acids are broken down, the carbon skeleton of the amino acids undergo oxidation to compounds that may enter the TCA cycle for further oxidation.

5. Glyoxylate Cycle:

- The glyoxylate cycle is used by some microorganisms when acetate is the sole carbon source or during oxidation of primary substrate that are converted to acetyl Co-A without the intermediate formation of pyruvic acid.
- This pathway does not occur in higher organisms because they are never forced to feed on 2- carbon molecules alone.
- The specific enzymes of the glyoxylate cycle are isocitrate lyase and malate synthase.
- The overall reaction of the glyoxylate cycle is:

$$2\ \text{Acetyl-CoA} \longrightarrow \text{Succinate} + 2H + 2\text{CoA}$$

- Acetyl Co-A enters the cycle at two places and condenses with oxaloacetate to give citrate, which is the entry point for the TCA cycle and the further reaction leads to the formation of isocitrate.

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- Isocitrate lyase is a splitting enzyme that produces succinate and glyoxylate.
- The second acetyl Co-A molecule condenses with glyoxylate to give malate by the action of malate synthase.

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Course Name	Agricultural Microbiology
Lesson 5	Microbial Metabolism: Energy Production by Aerobic Processes
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

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Learning Objectives

1. To develop a basic idea of tricarboxylic acid cycle in respiration.
2. To understand the energy yield in aerobic respiration.

1. TCA Cycle (Citric acid cycle or Krebs cycle):

- The TCA cycle is the central metabolic pathway of the cell and is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or dicarboxylic acid.
- The tricarboxylic acid cycle (TCA cycle) is a sequence of reactions that generate energy in the form of ATP and reduced coenzyme molecules ($NADH_2$ and $FADH_2$).
- Many intermediates in the cycle are precursors in the biosynthesis of amino acids, purines, pyrimidines, etc. For example, oxaloacetic acid and α- ketoglutaric acid are amino acid precursors.
- Pyruvate is firstly carboxylated and converted into acetyl Co-A, which is the connecting link between glycolysis and TCA cycle and acts as a fuel for TCA cycle.
- Acetyl Co-A is a 2- carbon energy rich molecule which initiates TCA cycle and is condensed with a 4-carbon intermediate, oxaloacetate, to form citrate and to begin the 6- carbon stage.
- The citrate is isomerised to give isocitrate which is oxidised and decarboxylated twice to produce α- keto glutarate, then succinyl Co-A.
- During this, 2 NADH molecules are generated and 2 carbons are released from the cycle as CO_2.
- Succinyl Co-A is converted into oxaloacetate via formation of succinate, fumarate and L- malate.
- During 2 oxidation steps i.e., succinate to fumarate and 1- malate to oxaloacetate, $1FADH_2$& 1NADH produced. GTP is produced during conversion of succinyl Co-A to succinate.

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- Finally, the oxaloacetate is reformed and becomes ready to join acetyl Co-A to proceed further.
- Krebs cycle generates 2 CO_2 molecules, 3 NADH, 1 $FADH_2$ and 1 GTP molecules, for each acetyl Co-A molecule oxidized.

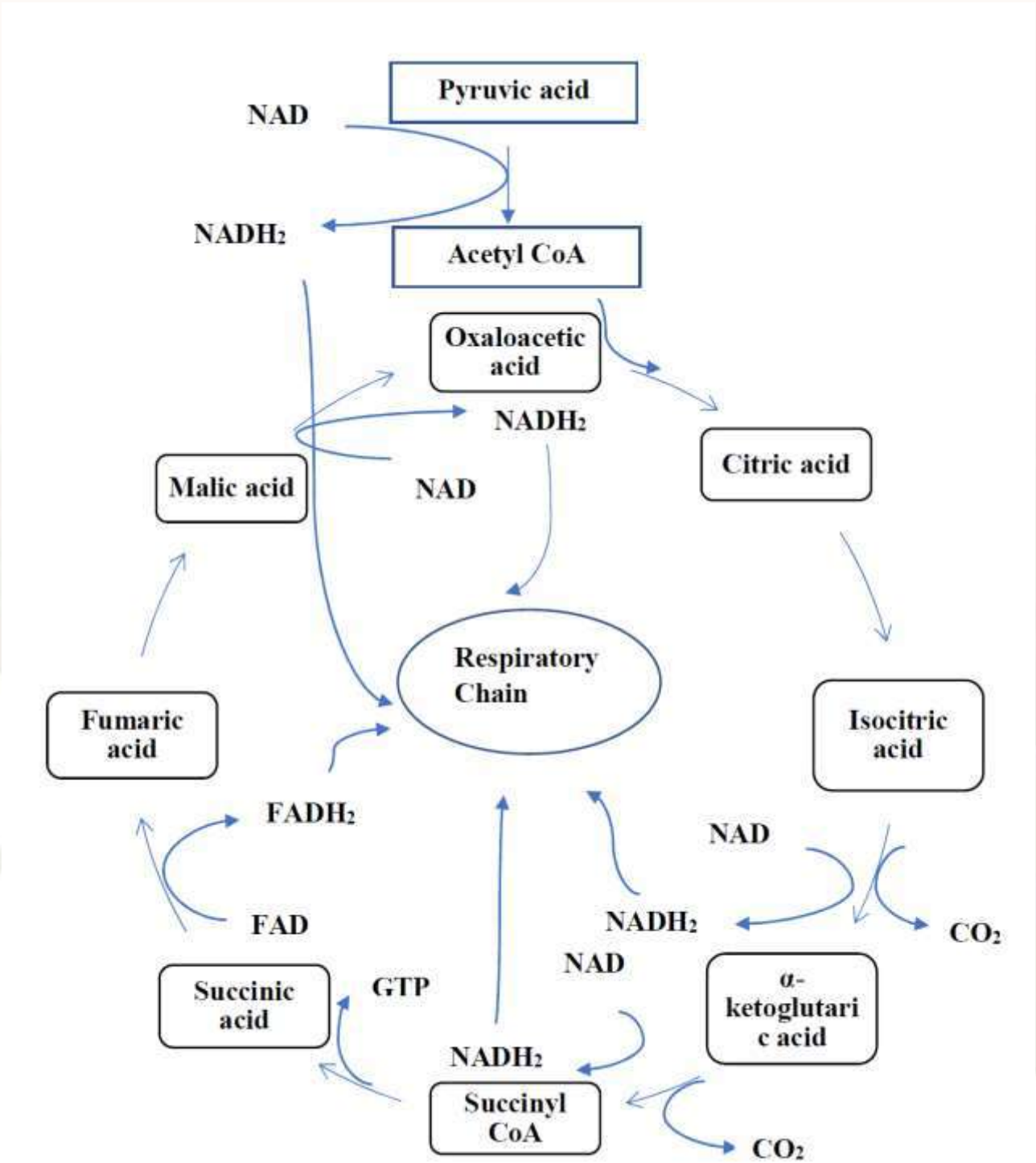

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Figure 1. The TCA cycle

- The generation of ATP from NADH and $FADH_2$ molecules associated with electron transport chain and oxidative phosphorylation.
- The generation of GTP molecule takes place via substrate level phosphorylation.
- The TCA cycle is an amphibolic cycle, which means that it functions not only in catabolic but also in anabolic (synthesis) reactions.

- Overall, reaction of the TCA cycle is

$$\text{Acetyl-CoA} + 3H_2O + 3NAD + FAD + ADP + Pi \rightarrow 2CO_2 + CoA + 3NADH_2 + FADH_2 + ATP$$

2. Energy yield in aerobic respiration

- For each glucose molecule broken down, there are 12 reduced coenzymes to be oxidized.
- 2 $FADH_2$ (I from each turn of TCA cycle) and 10 $NADH_2$ (2 from glycolysis; 2 from the gateway step between glycolysis and the TCA cycle and 6 from two turns of the TCA cycle).
- Since 3 ATP are produced from each NADH2 and 2 ATP from each $FADH_2$, there are 34 ATP generated from the reduced coenzymes via oxidative phosphorylation.
- The total yield of ATP from the aerobic respiration is 38 (34 from the oxidation of coenzymes, 2 from glycolysis and 2 from the side reaction of the TCA cycle).

3. Catabolism of Lipids

- Some organisms use lipid as an alternate source of energy.

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- Lipids are converted into intermediate of glycolytic and TCA pathways.
- Breakdown of lipids begins with the cleavage of triglycerides by the addition of water to form glycerol and fatty acids (Lipase enzyme).
- Glycerol can be converted to dihydroxyacetone phosphate which is broken down further.
- Fatty acids are oxidized by the successive removal of 2-carbon fragments in the form of acetyl CoA, known as β-oxidation.
- The acetyl CoA then enters the TCA cycle.

4. Catabolism of Proteins

- Bacteria secretes proteases to hydrolyse proteins to peptides.
- Peptidase that breaks down peptides to individual amino acids.
- When amino acids are broken down, the carbon skeleton of the amino acids undergo oxidation to compounds that may enter the TCA cycle for further oxidation.

5. Glyoxylate Cycle:

- The glyoxylate cycle is used by some microorganisms when acetate is the sole carbon source or during oxidation of primary substrate that are converted to acetyl Co-A without the intermediate formation of pyruvic acid.
- This pathway does not occur in higher organisms because they are never forced to feed on 2- carbon molecules alone.
- The specific enzymes of the glyoxylate cycle are isocitrate lyase and malate synthase.
- The overall reaction of the glyoxylate cycle is:

2 Acetyl-CoA ⟶ Succinate + 2H + 2CoA

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- Acetyl Co-A enters the cycle at two places and condenses with oxaloacetate to give citrate, which is the entry point for the TCA cycle and the further reaction leads to the formation of isocitrate.
- Isocitrate lyase is a splitting enzyme that produces succinate and glyoxylate.
- The second acetyl Co-A molecule condenses with glyoxylate to give malate by the action of malate synthase.

6. References

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Course Name	Agricultural Microbiology
Lesson 6	Energy Production by Photosynthesis and ATP Synthesis
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

Learning Objectives

1. To develop a basic idea of cyclic and non-cyclic photophosphorylation.
2. To understand the mechanism of ATP synthesis.

1. Introduction

- Plants, algae, and cyanobacteria are photoautotrophs and they use light as their source of energy and CO_2 as their sole source of carbon.
- In order for CO_2 to be useful for metabolism, it must first be reduced to carbohydrate and this process of conversion of CO_2 to carbohydrate in presence of light is called photosynthesis.
- The overall reaction can be written as;

$$2H_2O + CO_2 \xrightarrow[\text{chlorophyll}]{\text{In the presence of}} (CH_2O)_x + O_2 + H_2O$$

Carbohydrat

Here, $(CH_2O)_x$ is a formula representing any carbohydrate.

- Photosynthesis has two important requirements:
  1) A large amount of energy in the form of ATP, and
  2) A large quantity of a chemical reductant, in this case water

- Several groups of bacteria i.e., photoautotrophic green and purple bacteria also perform photosynthesis but unlike plants, algae, and cyanobacteria, they do not use water as their chemical reductant, nor do they produce O_2 as one of their end products of photosynthesis.
- The general equation for bacterial photosynthesis is:

$$2H_2A + CO_2 \xrightarrow[\text{chlorophyll}]{\text{In the presence of light and bacteria}} (CH_2O)x + 2A + H_2O$$

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Carbohydrat

Here, H_2A represents the chemical reductant, such as the inorganic compounds H_2, H_2S, or $H_2S_2O_3$, or the organic compounds lactate or succinate. If H_2A in this equation stood for H_2S, then A would stand for S.

2. Cyclic Photophosphorylation:

- It occurs in an oxygenic photosynthetic bacteria which possess chlorophylls, called bacteriochlorophyll, that differ from the chlorophylls of plants in structure and in light absorbing properties.
- Bacteriochlorophylls absorb light in the infrared region (725 to 1035nm) and they are not present in chloroplasts but are found in extensive membrane systems throughout the bacterial cell.
- When a molecule of bacteriochlorophyll absorbs a quantum of light, the energy of the light raises the molecule to an excited state in which an electron is given off and thus bacteriochlorophyll becomes positively charged and serves as an electron trap or strong oxidizing agent.
- The electron is transferred to an iron containing heme protein known as ferredoxin and from there it is passed successively to ubiquinone, to cytochrome b, and to cytochrome f, and finally back to the positively charged bacteriochlorophyll.
- The energy released in the step between cytochrome b and cytochrome f is used for photophosphorylation i.e., the generation of ATP from ADP and inorganic phosphate.
- In this process no $NADP^+$has been reduced and the reduction of $NADP^+$ in photosynthetic bacteria is accomplished not by

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photosynthesis but by using reducing power such as H2S and other inorganic and organic compounds.

- Essentially, the electron has gone around in a cycle, beginning with, and returning to, bacteriochlorophyll, so it is called cyclic photophosphorylation.

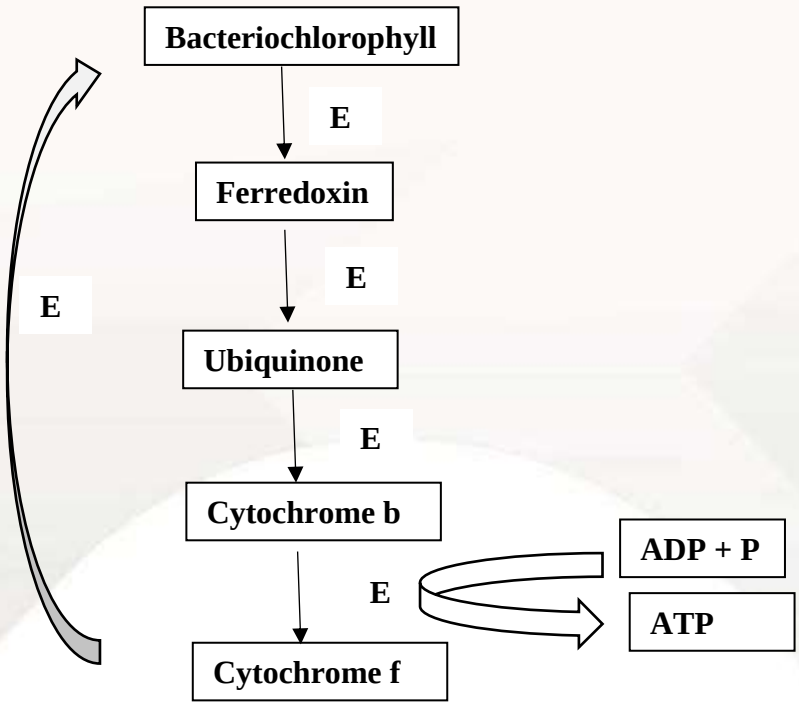

Figure 1. Cyclic photophosphorylation in oxygenic photosynthetic bacteria.

3. Non- cyclic Photophosphorylation:

- In plants, algae and cyanobacteria, non- cyclic photophosphorylation occurs in photosynthesis.
- In this process, when a molecule in pigment system II absorbs light, this energy raises the molecule to an excited state and the molecule releases an electron. This electron is transferred to plastoquinone, to cytochrome b, to cytochrome f, and finally to PS-I.

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- Photophosphorylation occurs with generation of ATP from ADP and inorganic phosphate in the step between cytochrome b and cytochrome f.
- When pigment system I absorbs light, it releases an electron and this electron is transferred from ferredoxin to flavoprotein, to $NADP^+$.
- Photophosphorylation occurs again between the release of the electron from PS-I to ferredoxin and $NADP^+$ is reduced in this process.
- This process differs from cyclic photophosphorylation because the electron lost by PS-II is not cycled back to it; instead, electrons are replaced in PS-II by the light generated breakdown of water, called photolysis.
- The protons necessary for reduction come from the dissociation of water, which results in evolution of O_2. Electrons are restored to the pigments of system II from the OH^- ion of H_2O.The OH^-ion is split to e^-, H^+, and ½ O_2 by photolysis.

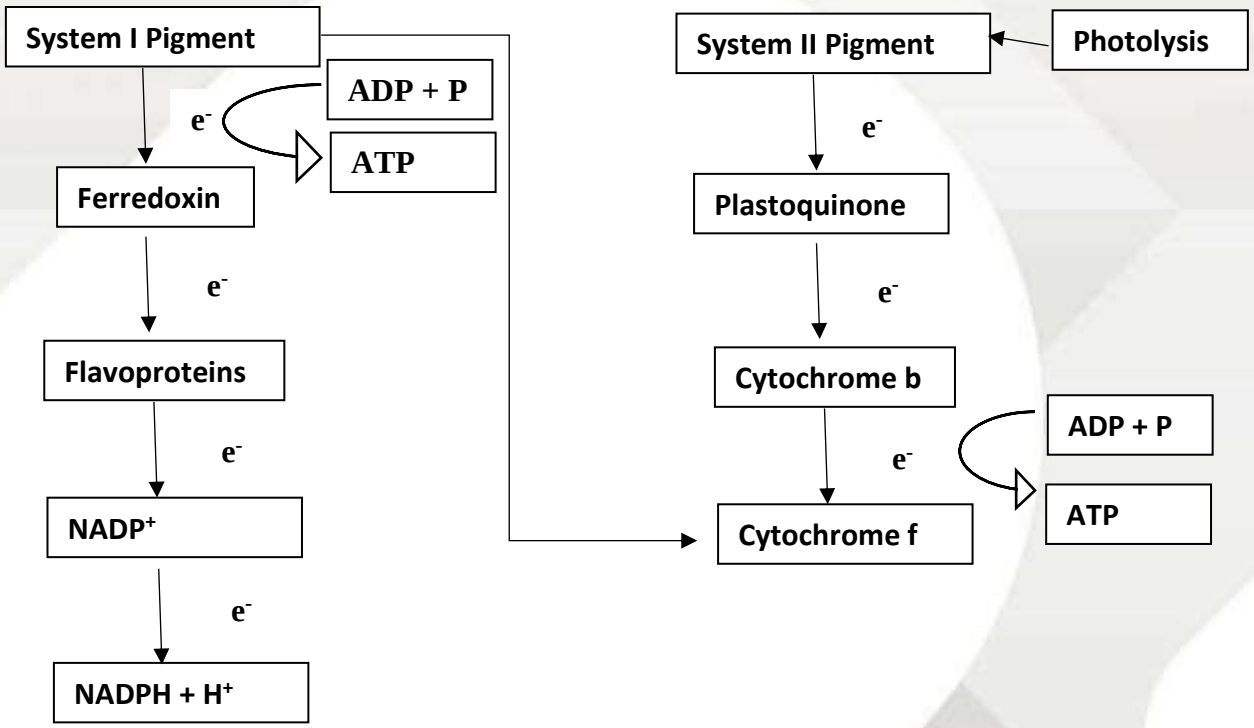

Figure 2. Non-Cyclic photophosphorylation in algae and cyanobacteria.

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4. The Mechanism of ATP Synthesis:

- The prevailing theory proposed to explain how energy released during electron transport is conserved in the form of ATP is the Chemiosmosis hypothesis advanced in 1961 by Peter Mitchell, a British biochemist. Mitchell was awarded the Nobel Prize for his work in this field in 1978.
- According to this theory, the flow of electrons through the system of carrier molecules releases energy which drives positively charged H^+ ions or protons across the membrane of chloroplasts, mitochondria, and bacterial cells.
- This movement of H^+ ions results in the acidification of the surrounding medium and the generation of a pH gradient across the cell membrane. In addition, it leads to the formation of an electric potential gradient.
- In this way, energy is released during the transfer of electrons through the respiratory chain is conserved as a proton motive force; the electric potential gradients are produced by pumping H^+ ions across the membrane.
- When the H^+ ions reenter the organelle or cell, they are transported by the membrane bound enzyme adenosine triphosphates. The energy released on reentry drives the synthesis of ATP.

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Course Name	Agricultural Microbiology
Lesson 7	Chemoautotrophs and Photoautotrophs
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

Learning Objectives

1. To comprehend the nature of nutrition in microbes.
2. To understand the differences between chemoautotrophs and photoautotrophs.

1. Introduction- Nutrition of microbes

- A nutrient is any substances used for the biosynthesis and energy production and the mode is known as nutrition.
- Bacteria also need nutrition for their sustenance specially to drive biochemical processes.
- Bacteria require sources of carbon, nitrogen, phosphorous, iron and a large number of other molecules.
- The nutritional requirements for bacteria can be grouped according to the carbon source and the energy source.
- Some types of bacteria must consume pre-formed organic molecules to obtain energy, while other bacteria can generate their own energy from inorganic sources.
- On the basis of energy source organisms are designated as: Phototrophs and Chemotrophs.
- The organisms which can utilize light as an energy source are known as phototrophs and gains energy from light; whereas, Chemotrophs gain energy from chemical compounds. They cannot carry out photosynthesis.
- On the basis of carbon source bacteria may be: Autotrophs and Heterotrophs.
- Autotrophs use CO_2 as their major or even sole source of carbon; whereas, heterotrophs require organic compounds as their carbon source.

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2. Chemoautotrophs

- Chemotrophs are a class of organisms that obtain their energy through the oxidation of inorganic molecules.
- The most common type of chemotrophic organisms is prokaryotic and include both bacteria and fungi. All of these organisms require carbon to survive and reproduce.
- The ability of chemotrophs to produce their own organic or carbon-containing molecules differentiates these organisms into two different classifications–chemoautotrophs and chemo heterotrophs.
- Chemoautotrophs derive energy for their life functions from inorganic chemicals. They feed on chemicals that are good electron donors, such as hydrogen sulphide, sulphur, or iron.
- Chemoautotrophs are able to “fix” carbon. They take atoms of carbon from inorganic compounds, such as carbon dioxide, and using it to make organic compounds such as sugars, proteins, and lipids.
- Chemoautotrophs are commonly found in environments where plants cannot survive, such as at the bottom of the ocean, or in acidic hot springs.

2.1. Examples of chemoautotrophs

2.1.1 Nitrifying bacteria

$$2NH_4^+ + 3\,H_2O \longrightarrow 2\,NO_2^- + 2\,H_2O + 4H^+ + \text{Energy}$$

- Ammonium ion oxidizes in the presence of oxygen to form nitrite with the release of energy. This energy is utilized by microbes to synthesize their foods.
- The microbes responsible for this process is *Nitrosomonas*.
- In the second step, this nitrite formed undergoes oxidation to form nit $2NO_2^- + O_2 \longrightarrow 2\,NO_3^- + \text{Energy}$

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- Nitrobacteria is responsible for carrying out the second step forming nitrate ions along with energy.

2.1.2. Hydrogen bacteria

- Hydrogen bacteria uses molecular hydrogen to oxidise it to water with the release of energy.

$$2H_2 + O_2 \longrightarrow 2\ H_2O + \text{Energy}$$

- Example includes *Hydrogen Monas*, *Pseudomonas saccharophylla*.
- When hydrogen disappears from the system, they get back to heterotrophic life.
- Hydrogen bacteria are usually facultative in nature.

2.1.3. Sulphur bacteria

- Sulphur bacteria oxidises free sulphur or sulphur compounds to generate energy for the synthesis of foods.

$$2H_2S + O_2 \longrightarrow 2S + 2H_2O + \text{Energy}$$

- When free sulphur is there in the environment, these bacteria oxidise it to sulphuric acid with the help of water and thereby releasing energy.

$$S + H_2O + O_2 \longrightarrow H_2SO_4 + \text{Energy}$$

- Example of sulphur bacteria: *Thiobacillus*, *Beggiatoa*.

2.1.4. Iron bacteria

- Iron bacteria are a type of bacteria that obtain energy by oxidizing ferrous iron which is dissolved in water.

$$Fe(HCO_3) + H_2O + O \longrightarrow 2Fe(OH)_2 + 4CO_2$$

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- In the above example, ferrous hydrogen carbonate gets oxidised to ferric hydroxide with the help of these bacteria.

- Example: *Ferro bacillus* and *Leptothrix*.

3. Photoautotrophs

- Photoautotrophs are those organisms that absorb light energy to produce complex organic compounds like carbohydrates and acquire energy.
- These bacteria capture the energy of sunlight and transform it into the chemical energy.
- In this process, CO_2 is reduced to carbohydrates.
- The hydrogen donor is water and the process produce free oxygen.
- Photoautotroph has chlorophyll pigment in the cell and its main function is to capture sunlight e.g., *Cyanobacteria*.
- Some photoautotrophic bacteria are anaerobes and have bacteriochlorophyll and bacteriovirdin like pigments in them.
- Photoautotrophs include green plants and photosynthetic bacteria; these organisms are also called holophytic.
- The photoautotrophs are also known as photosynthetic organisms which are of two types: Oxygenic and an oxygenic.
- Oxygenic photosynthetic organisms – they use chlorophyll to capture light energy and to oxidize water and break it into molecular oxygen. Anoxygenic photosynthetic organisms – they have a substance called bacteriophyll which captures light energy by absorbing at non-optical wavelengths.

3.1. Examples of photoautotrophs

3.1.1. Cyanobacteria

- The most well known photoautotrophic bacteria are cyanobacteria.
- Cyanobacteria are the only prokaryotes that perform oxygenic photosynthesis.

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- They can do this because they have cellular organelles very close in structure to plant chloroplasts.

3.1.2. Purple Sulphur Bacteria:

- These bacteria have the pigment bacteriochlorophyll located on the intracytoplasmic membrane i.e., thylakoids.
- These bacteria obtain energy from sulphur compounds e.g., *Chromatium. Theopedia rosea, Thiospirilium*.

3.1.3. Green Sulphur Bacteria:

- These bacteria use hydrogen sulfide (H_2S) as hydrogen donor.
- The reaction takes place in the presence of light and pigment e.g., *Chlorobium limicola, Chlorobacterium* etc.
- These bacteria take hydrogen from inorganic sources like sulphides and thiosulphates. Therefore, these bacteria are also known as photolithographs.

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Course Name	Agricultural Microbiology
Lesson 8	Understanding of Bacteriophages, Viroid's and Prions
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

Learning Objectives

1. To elaborate the idea on viruses of bacteria and their life cycle.
2. To describe unique characteristics of viroid's and prions.

1. Introduction:

- Bacteriophages are viruses that infect bacteria.
- Viruses are infectious agents so small that they can only be seen at magnification provided by the electron microscope.
- They are 10 to 100 times smaller than most bacteria, with an approximate range of 20 to 300 nm and due to this they can pass through the filters which do not permit the passage of most bacteria.
- Viruses are incapable of independent growth in artificial media. They can grow only in animal or plant cells or in microorganisms.
- Viruses are obligate intracellular parasites as they reproduce in cells by replication.
- Viruses largely lack metabolic machinery of their own to generate energy or to synthesis proteins. They depend on the host cell to carry out their vital functions.

1.1. Discovery and significance:

- Bacteriophages were discovered independently by Frederick W. Twort in England in 1915 and by Felix d'Herelle at the Pasteur Institute in Paris in 1917.
- Twort observed that bacterial colonies sometimes underwent lysis (dissolved and disappeared) and that this lytic effect could be transmitted from colony to colony.
- Even high dilutions of material from a lysed colony that had been passed through a bacterial filter could transmit the lytic effect. However, heating the filtrate destroyed its lytic property. From these observations, Twort cautiously suggested that the lytic agent might be a virus.

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- D'Herelle rediscovered this phenomenon in 1917 (hence the term Twort-d'Herelle phenomena) and coined the term bacteriophage, which means bacteria eater.
- Since bacteriophages are the smallest and simplest biological entities known which are capable of replication, they have been widely used in genetic research.
- The bacterium – bacteriophages interaction has become the model system for study of viral pathogenicity, thus provides better understanding of plant and animal infections with viral pathogens.

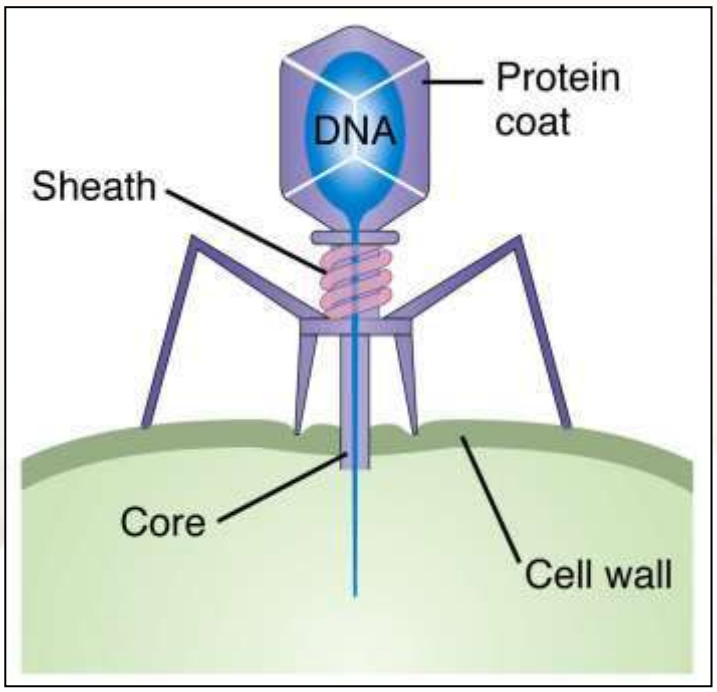

Figure 1 Bacteriophage (Source: https://www.mun.ca/biology/scarr/Bacteriophage.html)

2. General characteristics of bacteriophages:

- Widely distributed in nature, phages exist for most, if not all bacteria.
- Like all viruses, they are composed of a nucleic acid core surrounded by a protein coat.
- Occurs in different shapes, although many have a tail through which they inoculate the host cell with viral nucleic acid.

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2.1. Morphology and structure:

- The electron microscope has made it possible to determine the structural characteristics of bacterial viruses. Bacteriophage consists of a hexagonal head and a tail.
- All phages have nucleic acid core covered by a protein coat or capsid, which is made up of morphological subunits called capsomeres. The capsomeres consist of a no. of protein subunits or molecules called promoters.
- Most phages occur in one of two structural forms, having either cubic or helical symmetry.
- Cubic phages are regular solids or, more specifically, polyhedral; helical phages are rod shaped. Polyhedral phages are icosahedral in shape.

3. Replication of bacterial viruses (bacteriophages):

- The phage will attach to the bacteria by tail fibres.
- The sheath contracts driving the tail core into the cell wall and membrane.
- The virus injects its DNA.
- Then the phage replicates inside the cell by utilizing the host cells biosynthetic machinery about 25 minutes after initial infection, some 200 bacteriophages will be produced and they come out by breaking bacterial cell.

4. Lytic and Lysogenic Cycle

There are two main types of bacterial viruses (phages):

1) Lytic or virulent, and
2) Temperate or virulent

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Lytic phages:

When lytic phages infect the bacteria, the bacteria respond by producing large no. of viruses. At the end of the incubation period the host cell bursts or lyses releasing new phages to infect other host cells.

Temperate phages:

In the temperate type, the viral nucleic acid gets integrated with bacterial DNA and replicated in the host bacterial cells from one generation to another without any cell lysis.

4.1. Lytic cycle:

The lytic cycle of these bacteriophages can be divided into several stages which are as follows:

1) Adsorption:
   - The first step in infection of a host bacterial cell by phage is adsorption and infection of a host bacterial cell cannot occur without adsorption.
   - The tip of the virus tail becomes attached to the cell via specific receptor sites on the cell surface.
   - Attachment is specific in that certain viruses and susceptible bacteria have complementary molecular configurations at their opposing receptor sites.
   - In some cases, the specific receptor of the bacterium is part of the bacterial lipopolysaccharide, although any surface structure can function as a specific phage receptor including flagella, pili,etc.
2) Penetration:
   - If too many phages are attached to the bacterium and penetrate it, there may be premature lysis, which is not accompanied by the production of new virus.

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- This penetration is facilitated by localized digestion of certain cell surface structure either by phage enzyme (lysozyme) carried on the tail of the phage or by viral activation of host degradative enzyme.

3) Transcription:
   - Bacterial m-RNA and bacterial proteins stop being synthesized within a few minutes after entry of phage DNA.
   - Bacterial DNA is quickly degraded to small fragments and the nucleoid region of the bacterium becomes dispersed. Some phage m-RNA is made immediately after infection.

4) Assembly and Release:
   - Only after the synthesis of both structural proteins and nucleic acid, phage components begin to assemble into mature phages.
   - About 25 min after initial infection, some 200 new bacteriophages are assembled and the bacterial cell bursts, releasing the new phages to infect other bacteria.

4.2. Lysogenic cycle:

- In lysogeny, the viral DNA of the temperate phage instead of taking over the functions of the host cell genes, it is incorporated into the host DNA and becomes a prophage in the bacterial chromosomes acting as a gene.
- In this the bacterial cell metabolizes and reproduces normally and the viral DNA is transmitted to each daughter cell in successive generations.
- In the lysogenic state, the virus is simply one of the bacterial genes.

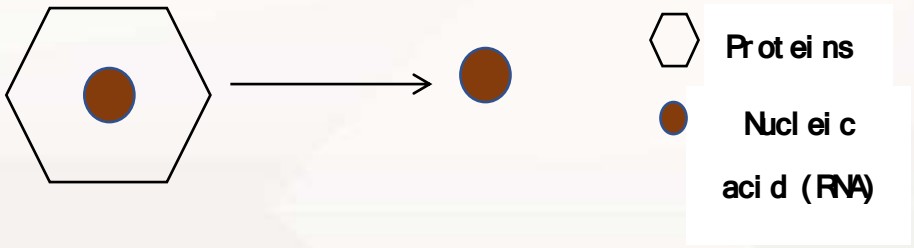
4.2.1. Mechanism of lysogeny:

- When a sensitive bacterium is infected by a temperate phage, two things may happen. In some of the infected cells, multiplication of the phage occurs and a lytic cycle takes place. In the other infected cells, the multiplication of phage is repressed and lysogenization occurs.
- The temperate phage possesses a gene that codes for a repressor protein which makes the cell resistant to lysis.

- The repressor protein reacts with two different sites to prevent the expression of phage lytic functions and formation of mature phage particles.

5. Viroid's

- Viroid's are smallest known pathogens.
- It is covalently closed, circular, single-stranded RNA molecules of low molecular weight that multiply in plants and cause diseases.



- Viroid's were discovered by Diener (1971) as a strange and unique type of plant pathogen, associated with potato spindle tuber disease, which is he termed Viroid.
- The RNA cannot code for a protein and therefore, its mode of replication and pathogenesis are baffling.

Despite the similarity in name, the viroid differs from viruses in many ways;

- Viroid's are much smaller
- Do not have a protein coat
- Have no extracellular existence
- Have no coding capacity for protein
- The genome does not behave as messenger RNA or its compliment

5.1. Size and structure

- The molecular weight of viroid ranges between 100,000 to 140,000 daltons (d), which is much less than the smallest viruses.

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- Till date, 30 viroid's have been identified, ranging in length between 120-475 nucleotides.
- Viroid's differ from the virus in structure and form. These consist of solely short strands of circular and single-stranded RNA without the protein coats.
- The excessive base pairing gives it a rod-like shape. On heating, the viroid is denatured and the straight structure changes to circle.

5.2. Classification

The I.C.T.V (2005, 8th Report) classifies viroid as sub-viral agents, under two families: Pospiviroidae and Avsunviroidae depending

- on the site of their replication
- nucleotide sequences
- structural homologies

5.3. Pathogenesis

How viroid's induce symptoms without encoding any protein, has long been a matter of confusion. Some of the probable mechanisms are:

- Inhibition of protein synthesis by viroid induce protein kinase
- RNA silencing
- Faulty RNA processing

5.4. Origin of Viroid's

- A number of features of viroid's suggest that they may have originated in the hypothetical prebiotic RNA world.
- They can possess a ribosomes activity.
- They are GC rich which would attenuate the low fidelities of replication activities.
- They are circular and so they do not require start and stop codons for replication.

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- They move within the plant through phloem and plasmodesmata to become systemic. They seem to form a quasi -species population and can recombine.

6. Prions

- Prions are infectious misfolded proteins and also known as proteinaceous infectious particle.
- Prions, so-called because they are proteinaceous, are infectious particles, smaller than viruses, that contain no nucleic acids (neither DNA nor RNA).
- Prions were discovered by Prusiner (1982), during the research for the cause of scrapie, a neurological disease of sheep. Similar neurological disorders occur human beings also viz., kuru, CJ and GS disease.
- Before the discovery of prions, these diseases were thought to be caused by a “slow virus”. Prions are normal protein present commonly in the cell membrane of neuron cells.
- All these diseases show presence of vacuoles, or fluid filled cavities, containing clumps of proteins (prions) in the brain cells. These proteins are called PrP (protease-resistant protein).
- In normal conditions, these proteins are present in the membranes of the nerve cells. The PrP are resistant to protease and this leads to their accumulation.
- Prions are normal proteins folded abnormally due to defect in the gene produces PrP. These PrP formed by the defective gene, has proline in place of leucine.

6.1. Replication of Prions

- PrP exists in two forms: PrP^{c}, the normal form of the protein, and PrP^{sc}, the infectious form.
- Once introduced into the body, the PrP^{sc} contained within the prion binds to PrP^{c} and converts it to PrP^{sc}.

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- This leads to an exponential increase of the PrP^{SC} protein, which aggregates. PrP^{SC} is folded abnormally; the resulting conformation (shape) is directly responsible for the lesions seen in the brains of infected cattle.

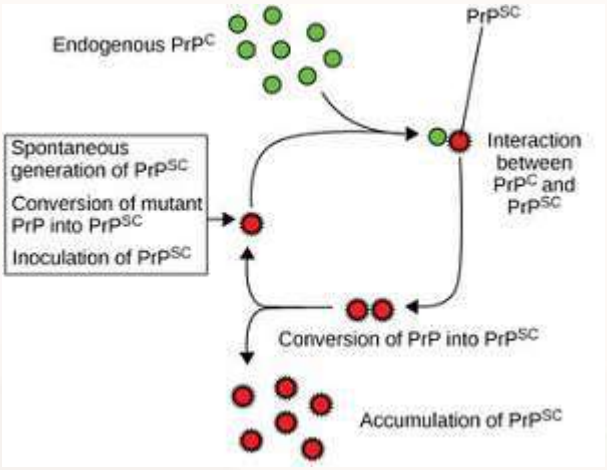

Figure 2. Endogenous normal prion protein (PrPc) is converted into the disease-causing form (PrPsc) when it encounters this variant form of the protein. (https://courses.lumenlearning.com/boundless-biology/chapter/prions-and-viroids/)

6.2. Classification

ICTV (2005) classifies prions under subviral agents, along with viral satellites and viroid's. The prions are put under 2 types and 2 subgroups (which are not regular taxa) as follows.

- Type: Mammalian prions; Subgroup: Scrapie agent
- Type: Fungal prions; Subgroups: URE3 (host fungi)

Course Name	Agricultural Microbiology
Lesson 9	Bacterial Genetics and Gene Regulation
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

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Learning Objectives

1. To get acquainted with the types of bacterial recombination.
2. To understand the prokaryotic gene regulation.

1. Introduction

- Like of biochemical principles, genetic principles are universal. The study of microbial genetics has contributed much to what we know about the genetics of all organisms.
- Bacterial cultures millions of individual cells. Therefore, by using selective techniques, rare genetics events can be discovered.
- The very advantage of studying bacteria is that we can identify species and even strains capable of transmitting genetic information from generations to generations with great accuracy.
- Any changes in genetic relates to genotypes and phenotypes. Genotypes refers to the genetic constitution of the cell and phenotype represents the heritable total potential characteristics of a cell.
- Phenotype = Genotype X Environment
  E.g. A facultative anaerobe will produce different end product of metabolism, depending on the presence or absence of oxygen. It means a single organism produces different metabolic products just because of the variation in oxygen.

2. Genotypic changes

- Genotype of cell is determined by the information contained in the chromosome / chromosomes.
- Chromosomes are divided into genes.
- Gene is a functional unit of inheritance; it specifies the formation of a particular polypeptide as well as various types of RNA.
- Gene consists of nucleotide pairs.

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- Any gene is a capable of changing or mutating to different form so that it specifies formation of an altered or new protein which may change the characteristics of cell (sometimes leads to death).

2.1. Mutation

- Any change in nucleotide sequence of a gene.
- There are two types of mutation:

  1. **Point mutation**- It occurs as a result of the substitution of one nucleotide for another in the specific nucleotide sequence in a gene. The substitution of one purine for another purine or one pyrimidine for another pyrimidine is termed ***transition***. A ***Trans version*** id the replacement of a purine by a pyrimidine, or vice versa.

  2. **Frameshift mutation**- These mutations result from an addition or loss of one or more nucleotides in a gene and are termed insertion and deletion mutation respectively.
- Mutations most commonly occur during DNA replication.
- Any agent that increases the mutation rate is called a mutagen.
- Mutation are of two types: *Induced* (used by mutagens) and *Spontaneous*.
- Mutagenic chemicals are there to induce mutation

  1. Compounds that can react chemically with DNA. e.g. Nitrous acid.

  2. Base analogs- similar to structure to normal DNA base and can replace.

  3. Intercalating agents- Flat molecules that slip in between base pairs in the central stack of the DNA helix.
- Mutations can occur because of transposons. Transposon are unit of DNA which move from one DNA molecule to another, inserting themselves nearly at random.
- Mutation are repaired through photo reactivation where cells are exposed to lethal doses of UV are immediately exposed top visible light. Some bacteria have enzymes called endonucleases and

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exonucleases that excise or cut out a damaged segment of DNA. Other enzymes like polymerases and ligases repair the resulting break by filling in the gap and joining the fragments together.

- Mutation rate is defined as the average number of mutations per cell division. Mutation rate of any single gene ranges between 10^{-3} to 10^{-9} per cell division.
- Suppose if a mutation rate for X component is 10^{-8} per cell division and for that of Y is 10^{-6} per cell division, then the probability that both mutation occurs will be 10^{-14}.
- Reverse mutation- many mutants are able to revert back to its wild type condition is reverse mutation.

3. Bacterial Recombination

- It is the formation of new genotype by reassortment of genes following an exchange of genetic materials between two chromosomes which have similar genes at corresponding sites.
- In general, there are three types of gene transfer:

  1. Conjugation- Transfer of genes between cells that are in physical contact with each other.

  2. Transduction – Transfer of genes from one cell to another by a bacteriophage.

  3. Transformation- Transfer of cell free or naked DNA from each cell to another.

3.1. Conjugation

- A clear understanding of conjugation comes with the discovery of sexual differentiation in E. coli (different mating types of bacterium exists).
- Male cell contains small circular piece of DNA in cytoplasm (not in chromosome) known as sex factor or the F factor (F^+) and female lacks this feature (F^-).
- F^- x F^- do not yield recombinants.

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- On F+ x F- cross, male replicates its sex factor, and one copy of it is always transferred to the female recipient. The F^- cell is converted to an F^+ cell and is capable of serving as a donor.
- This shows that there is 100% F transfer during cross of F^+ and F^-, but the formation of recombinant occurs at a low frequency about one recombinant per 10^4 to 10^5 cell. It proves transfer of F factor is independent of transfer of chromosomal genes.
- Since, the transfer of F factor is independent, it follows that the F factor DNA replicates independently of the F^+ donor cell's normal chromosomes. The F factor DNA is only sufficient to specify about 40 genes which control sex-factor replication and synthesis of sex pili.
- One or more sex pili are produced by each F^+ cell. Sex pili seems to bind an F^- cell to an F^+ cell and then to retract into the F^+ cell, pulling the F^- cell into close contact. There is evidence that sex pili are tubules through which DNA passes from an F^+ to an F^- cell during conjugation.

3.2. Transduction

- This was discovered by Zinder and Lederberg in 1962.
- Bacteriophage acts a vector in transduction. Bacteriophage undergoes a rapid lytic growth in the host cell. It then injects their DNA into the bacterium. Replication process of DNA is faster in phage.
- However, some bacterial viruses do not lyse the cell, carry DNA that can behave as a episome in bacteria, such as F factor and can integrate into bacterial genome (known as prophage).
- This phage particle may become filled with cell chromosomal DNA or a chromosomal and phage DNA. Such aberrant pages can attach to other bacteria and introduce bacterial DNA from one cell to another.
- Thus, bacterial transduction is the transfer by a bacteriophage serving as a vector.

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3.3. Transformation

- In 1928, Griffith injected mice with a mixture consisting of few (non-capsulated and non- pathogenic) pneumococci and a large number of heat killed smooth (capsulated and pathogenic) cells.
- The mice subsequently died of pneumonia and live smooth cells were isolated from their blood.
- Apparently, some factor responsible for the pathogenicity of the smooth bacteria (even though they were dead) had been transferred to the living rough bacteria and had transformed them into pathogenic smooth ones.
- Griffith showed that the transforming factor could be passed from the transformed cells to their progeny and thus had the characteristics of a gene.
- This transforming principle was identified as DNA by Avery, MacLeod and McCarty in 1944.
- So, in transformation naked DNA containing small amount of genetic information is transferred from one bacterium to another.

4. Gene Expression

- The regulation of gene activity is best controlled at the level of gene transcription. One can better understand the regulation of gene expression in prokaryotes by discussing lac operon concept in *E.coli*.
- The lactose or lac operon of *Escherichia coli* is a cluster of three structural genes encoding proteins involved in lactose metabolism and the sites on the DNA involved in the regulation of the operon.
- Many protein-coding genes in bacteria are clustered together in operons which serve as transcriptional units that are co-ordinately regulated.
- It was Jacob and Monod in 1961 who proposed the operon model for the regulation of transcription.
- The *lac* operon contains three genes: *lacZ*, *lacy*, and *lacA*. These genes are transcribed as a single mRNA, under control of one promoter.

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- Genes in the *lac* operon specify proteins that help the cell utilize lactose. *LacZ* encodes an enzyme that splits lactose into monosaccharides (single-unit sugars) that can be fed into glycolysis. Similarly, *lacY* encodes a membrane-embedded transporter that helps bring lactose into the cell.
- In addition to the three genes, the *lac* operon also contains a number of regulatory DNA sequences designated as i (repressor gene), p (promoter) and o (operator). These are regions of DNA to which particular regulatory proteins can bind, controlling transcription of the operon.
- Transcription of the operon is both negatively and positively controlled.
- Negative control is mediated by the lac repressor which binds to o gene and blocks transcription. Inducers such as lactose stimulate lac mRNA synthesis by binding to the repressor and reducing its affinity for the operator. Both repression and induction of enzyme synthesis are negative control system because in either case the synthesis of enzyme can proceed only when the repressor is removed from its blocking site on the o gene.
- Positive control of enzyme synthesis is said to occur when an association between a protein and a part of the regulatory region of an operon is essential for expression of related structural genes in the operon. Expression of the lac operon is inhibited when a more efficient source of energy such as glucose is present in the medium. The presence of glucose results in a decreased concentration of intracellular AMP. Cyclic AMP is needed for efficient expression of the lac operon since it activates the catabolite gene activator protein (CAP), which in turn activates transcription of lac mRNA by RNA polymerase at the promoter site.
- This model gives an insight into the molecular event of gene regulation. It shows the precision by which regulatory proteins modulate gene function: the repressor must recognise the specific nucleotide sequence of the operator gene on the one hand, and on the other hand they must recognise specific inducer molecules like lactose.

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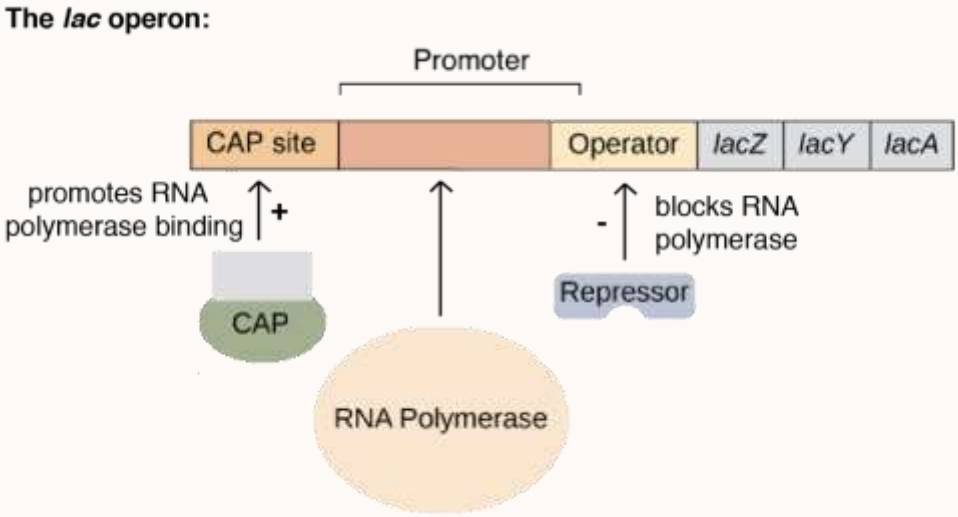

Figure 1. The Lac Operon

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Course Name	Agricultural Microbiology
Lesson 10	Concepts of Genetic Engineering and Genetically Modified Organisms
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

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Learning Objectives

1. To get acquainted with recombinant DNA technology.
2. To describe the techniques of producing genetically modified organisms.

1. Genetic engineering

- DNA is a genetic material which contains all hereditary information and the primary function of DNA is to makes proteins. DNA is transcribed into mRNA and mRNA is translated into protein.
- The specific regions on the DNA molecule that direct the synthesis of proteins are called genes. By changing the nucleotide sequence in DNA, the protein formation is changed.
- Recombinant DNA (rDNA) is taking a piece of DNA and combining it with another strand of DNA. RDNA is also referred as chimera DNA.
- By combining two or more strands of DNA from two different organisms, one can create a new strand of DNA.
- Recombinant DNA Technology (rDNA Tech) or genetic engineering is concerned with the manipulation of genetic materials towards desired end in a directed way. It is also known as gene cloning. Genetic engineering aims at isolating DNA segments of one organism of interest and fining that with DNA of second unrelated organisms.
- Genetic Engineering is a term used to refer the manipulation of existing genes with the new or foreign genes isolated from other organisms. In other words, it is the manipulation of genetic architecture of an organism using recombinant DNA technology.

- It is the manipulation of genetic makeup of an organism for desired phenotype. Genetic engineering is done by the recombinant DNA process (rDNA).

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- The DNA which constitutes the genetic alphabet of all living organisms and contributes to the vast diversity evident in all living beings. The basis of molecular genetics is the process of genetic recombination, the breakage and reunion of DNA molecules (restriction endonuclease and ligases) which is the fundamental importance to all living organisms as a mechanism for adaptation and variation.
- An organism that is generated through genetic engineering is considered to be genetically modified (GM) and the resulting entity is a genetically modified organism (GMO).

2. Chronological achievements of Genetic Engineering

- 1970 Smith and Nathans discovered the restriction enzymes that cut a DNA molecule into smaller fragments.
- 1972 Berg and others combined DNAs from two viruses to produce what is called recombinant DNA (rDNA).
- 1973 Cohen and Boyer inserted recombinant DNAs into host bacteria.
- 1977 Genetech, one of the first genetic engineering companies established.
- 1977 Sanger and Gilbert independently discovered techniques for rapid sequencing of nucleotides in DNA molecules.
- 1982 Human insulin produced by rDNA is marketed under the trade name ‘Humulin’.
- 1983 Tracy Moreno, a ten-year-old girl born with growth hormone deficiency, grows 5 inches in one year of treatment with engineered hormones.

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- 1997 Scientists remove the DNA-containing nucleus from a female's egg and replace it with a nucleus from a different animal of the same species. The result, first demonstrated by the birth of a cloned sheep named Dolly.

3. Steps in producing genetically engineering bacterium

The essential steps in the technology of producing a genetically modified bacterium are as follows:

- Source of donor genetic material: DNA containing the genetic code for the property to be transferred into a bacterium is isolated from cells, or it may be synthesized. The DNA is tailored to form the gene which contains the genetic information to code for a desired characteristic such as production of human insulin.
- Production of hybrid DNA molecule: The donor genetic material (DNA segment) is incorporated into the DNA molecule of bacteriophage or a bacterial plasmid. This is accomplished by the use of two enzymes: restriction endonuclease and ligases. Restriction endonucleases cut double stranded DNA molecules at particular nucleotide sequence. In this process both the donor DNA and the agent into which the fragment of the donor DNA is to be incorporated are treated with the same restriction endonuclease. The fragments can be connected by the addition of an enzyme called DNA ligase.
- Incorporation of hybrid DNA into host cell: **Transformation** in genetic engineering is the process by which plasmid hybrid DNA molecules introduced into a competent host bacterial cell. **Transfection** involves the introduction of phage hybrid DNA into the host cell. Transformation depends on treating the recipient bacteria with calcium chloride to make the membrane permeable to DNA.
- When bacteria are transformed or transfected, a mixture of various genotypes is usually produced. Each cell is capable of binary fission

yielding colony of identical cell. Once a colony is identified, the bacteria can be grown in limitless quantity.

- Cloning is the isolation and purification of individual genetically unique cells; the progeny of selected bacterium constitutes a clone and the gene is said to have been cloned.
- It is possible to obtain the DNA of the cloned gene in pure state and in unlimited amounts. Even, one plasmid inserted into E. coli may generates a hundred or more copies of itself within the cell.

4. Risk of Genetic Engineering

Although, genetic engineering contributes to the improvement of our health, environment and many aspects of lives, but still there are risks associated with.

- Spread of new diseases: New dangerous forms of microorganisms can be developed

  Through recombinant DNA technology either accidentally or laboratory through drainage lab glass ware, personal etc, may lead to spread and origin of new types of diseases which may pose a serious problem.
- Effect on evolution: Nature has provided several barriers for the exchange of DNA

  Between prokaryotes and eukaryotes. Recombinant DNA technology permits exchange

  Of DNA between these classes of organisms and thus interferes with natural process of

  Evolution.
- Biological warfare: There is a fear that genetic engineering technologies will be used

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For biological warfare. In such warfare disease carrying microorganisms can be used

Against enemy. This will lead to disaster.

Safety measures

Dangers of recombinant DNA technologies can be minimized with

- Increased experience and knowledge.
- By applying safer measures to check the escape of new microorganisms from

  Laboratories.

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Course Name	Agricultural Microbiology
Lesson 11	Soil Microbial Groups: Classification
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

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Learning Objectives

1. To recognize soil as a habitat for microorganisms.

2. To understand the classification of soil microbes in details.

1. Soil Microbes

- Soil microorganisms are broadly defined as a group of microscopic life forms that include bacteria, archaea, viruses, and eukaryotes like fungi.
- Most importantly, soil microbes mainly inhabit in soils.
- The role of soil microorganisms was recognized as early as 1838 when J.B. Boussingault first observed that legumes could utilize nitrogen from the atmosphere.
- This was further confirmed by M.W. Beijerinck when he isolated bacteria from root nodules of legumes which were the agents for nitrogen fixation.

1.1. Why they inhabit in soils?

- Oxygen of soil air trapped in pore spaces and dissolved in soil water is used for the aerobic organisms.
- Soil organic matter acts as the source of food for them.
- Soil contains essential nutrients needed for the protoplasmic growth as well as for carrying out metabolic activities.
- Soil contains water essentially required for microbes (95% of protoplasm).
- Soil possesses insulation as well as buffering capacity, so that microbes can sustain any adverse change therein.

2. Classification of Soil Microbes

There are several classifications of microbes are there based on different aspects.

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A) Winogradsky (1924) has divided soil organism into two group based on carbon sources available in soil.

- **Indigenous/Autochthonous: -** group of microbes in which multiplication (increase in cell number) and proliferation (increase in cell size) is dependent upon recalcitrant (resistant to degradation or non-labile pool of carbon) organic material for carbon source. Ex. Actinomycetes.
- **Zymogenous: -** Microbes which depends on non-recalcitrant source of carbon for their multiplication and proliferation, and called as zymogenous. Ex. Most of the bacterial species.

B) Paul and Clark (1996) studied the laboratory conditions and classified microorganism based on quantity of carbon source.

- **Pleiotrophic: -** microbes which are only able to multiply in abundance solubility of carbon at least upto 1000 mg Soluble carbon per litre of media.
- **Copiotrophic:** - microbes which can multiply under strong deficiency of soluble carbon, even lower up to 15 mg soluble carbon per Litre of media.

** Only 1 % microorganisms are identified or culturable remaining 99% are uncultivable microorganisms.

Metagenomics: it gives idea about total microbial population. Like genomics itself, metagenomics is both a set of *research techniques*, comprising many related approaches and methods, and a *research field*. In Greek, *Meta* means "transcendent." In its approaches and methods, metagenomics circumvents the unculturability and genomic diversity of most microbes.

2.1. Classification of soil organism on the basis of broad spectrum

Organisms present in soils are classified into two main groups:

- Soil flora- Plant Kingdom
- Soil fauna- Animal forms

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These are further divided into:

- Macro organisms- big enough to observe through naked eyes.
- Microorganisms- very tiny and needs microscope or advance instruments.

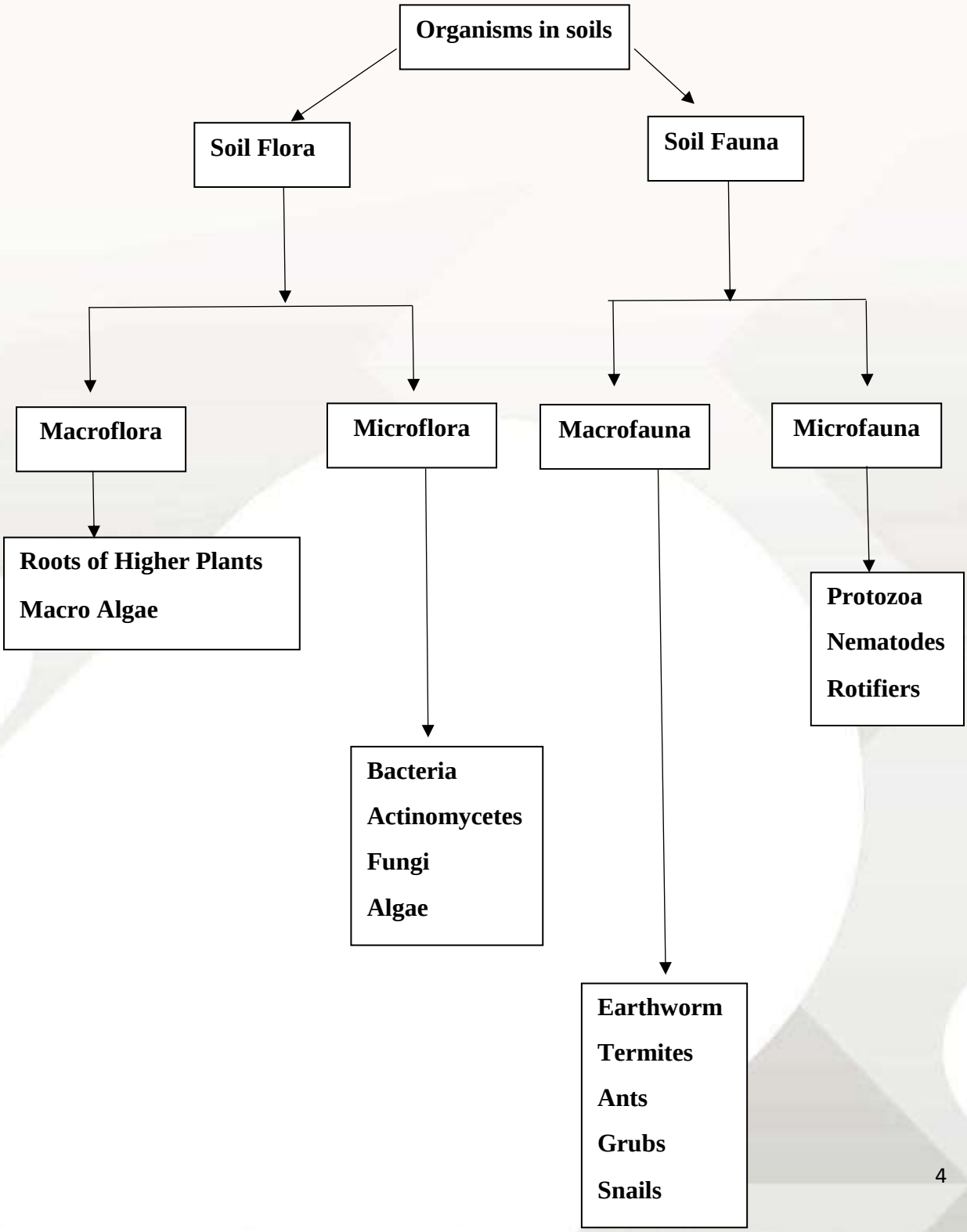

Figure 1. Classification of Soil Organisms

2.2. On the basis of carbon requirement, soil microbes can be classified into:

- Heterotrophs (most of bacteria comes under this): Derive their energy and carbon requirement from the complex organic compound e.g. Starch, carbohydrate, and protein. For example; *Rhizobium*
- Autotrophs: Drive their carbon and energy requirement from atmosphere or environment

  Under autotrophs, there are

  a) They derive their energy requirement from the process of photosynthesis or photosynthates and they are also known as photoautotroph.

  e.g., Cyanobacteria

  Purple sulphur bacteria

  Green sulphur bacteria

  b) They derive their energy requirement from the oxidation or reduction of inorganic compounds like nitrate, sulphate etc. (knowns as chemoautotrophs)

  e.g., Nitrifiers, *Pseudomonas*

2.3. On the basis of oxygen sensitivity, soil microbes can be classified as:

- Aerobes e.g., most of the populous bacteria
- Anaerobes e.g., *Clostridium*
- Facultative anaerobes (basically aerobic) e.g., *Pseudomonas*
- Microaerophilic- Grows in low oxygen concentration e.g., *Caulobacter*

2.4. On the basis of soil reaction

- Acidophilic: - Prefers acid condition; pH (3 to 4.5), e.g., *Beijerenckia*, *Derxia*

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- Halophyte: - Salt loving bacteria also used for the reclamation of salt affected; soil pH more than (8.5). e.g., *Halomonas*

2.5. On the basis of temperature

- Psychrophilic: - have optimum temperature for growth below 10℃; e.g., *Enterobacter*, *Listeria monocytogenes*
- Mesophilic: 25 to 37℃; e.g. S*taphylococcus aureus, E.coli*
- Thermophilic: - more than 45℃; e.g., Actinomycetes

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Course Name	Agricultural Microbiology
Lesson 12	Carbon Cycle
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

Learning Objectives

1. Understanding the plant composition and its possible decomposition process.

2. To develop a basic knowledge of the cycling of carbon across plants, animals and microorganisms.

1. Introduction:

- Carbon is one of the most important elements in biological system and component of all cell structures, which constitute about 50% of all living organisms.
- Soil microorganisms in true sense are one of the natural safety valves that regulate biogeochemical transformation in soil ecosystem.
- The various essential biological elements such as C, N, S, and P, apart from H and O, form the primary building blocks of cells, and their constituent elements are recycled through tiny microorganisms present in soil.
- The carbon cycle in microorganisms is part of a larger cycling of carbon that occurs in a global scale. Assimilation of carbon plays a pivotal role in the life cycle of plants and animals.
- It involves fixation of CO_2 from the atmosphere and its return to the atmosphere through a no. of microbial processes.
- The terrestrial C- cycle is dominated by the balance between photosynthesis and respiration.

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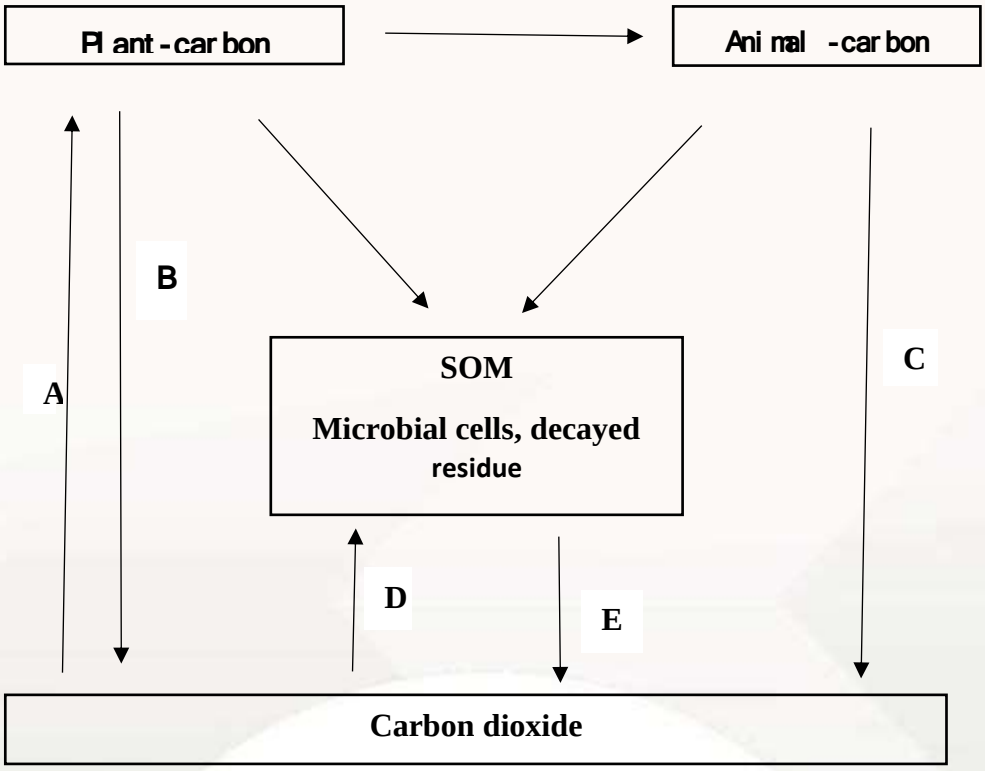
2. The carbon cycle:



In the above figure,

A- Photosynthesis
B- Respiration by plant
C- Respiration by animal
D- Autotrophic microorganism
E- Microbial Respiration

- The carbon cycle shows diagrammatically the fixation or immobilization of carbon in organic form from CO_2 and its return to the atmosphere as CO_2. Once fixed, the carbon is no longer available for body building of other organisms, unless this is mineralized to CO_2 by microorganisms.

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- Carbon is transferred from the atmosphere to soil by the process called photosynthesis carried out by carbon fixing autotrophic organisms, mainly plants, photo, and chemoautotrophic microbes that synthesize atmospheric CO_2 into organic material.
- The main source of organic matter received by soil is the plant residues and breakdown of this takes place through the activity of a number of soil microflora and micro fauna.
- The breakdown of the carbohydrate serves to supply energy to the microorganism. This process is known as respiration.
- Examples of autotrophic microorganisms- Cyanobacteria, Purple Sulphur bacteria, Green Sulphur bacteria, Nitrifies etc.

3. Major constituents of plant material:

- To understand the pathway of decomposition, it is necessary to know the major constituents of plant material.
- Plant carbon can be roughly characterized as follows:

1) Carbohydrates – 30 to 75% dry weight
   Cellulose – 15 to 60%
   Hemicellulose – 10 to 30%
   Sugars and starches – 1 to 5%
2) Lignin – 10 to 30 % dry weight
3) N – containing compounds i.e., proteins and amino acids – 1 to 5 % dry weight
4) Waxes and pigments – 1 %
5) Pectin -1%
6) Others – 5 to 20% dry weight

Fats, oils, organic acids and hydrocarbons

The value varies as plant ages, the cellulose, hemi-cellulose and lignin contents increase while the simple sugars, amino acids, proteins, fats and oil decreases.

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- Fresh carbonaceous organic material when added to soil are immediately attacked by soil microbes under favorable environment.
- The breakdown is basically enzymatic oxidation of carbon and hydrogen with the liberation of heat energy.

$$\text{Organic compounds} \xrightarrow{O_2} CO_2 + H_2O + \text{energy}$$

- Part of the carbon is assimilated by microorganisms for their body building and part of it is liberated as CO_2 gas goes back to the atmosphere.
- The rate of decomposition is rapid at the beginning but slows down gradually. All the plant constituents are not decomposed at the same rate.

  Amino sugars > starches > proteinaceous substance >hemicellulose >cellulose >lignin

4. Factors affecting the soil organic matter decomposition:

- The factors that govern microbial activity also influence the decomposition of organic matter in soil.
- These include
  1) Temperature
  2) Moisture
  3) pH
  4) oxygen availability
  5) C/N ratio of substrate
  6) Lignin content
  7) Soil type
  8) Clay content
  9) Management practices
- The amount of biomass and metabolite formation during the first phase of decomposition is greatly influenced by soil texture.

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- Clay soil retains more organic matter than sandy soil. Moreover, clay protect the organic matter through adsorption of readily available substrates so makes them less accessible to microbes.
- Decomposition process is 4 times faster in tropical areas than temperate.
- PH 6 to 8 is favorable for decomposition.
- C/N ratio is a vital factor in organic matter decomposition because intense competition for available N between microorganisms occurs when a residue of wide C/N ratio is added to soil.
- An optimum C/N ratio in the range 20 to 25 seems to be ideal for decomposition which bring about equilibrium between immobilization and mineralization.
- Generally, when residues with C/N > 20:1 are added to soil, soil N is immobilized during the initial decomposition process. For residues with C/N ratio < 20:1, there is release of mineral N early in the decomposition process.
- Microbes uses to maintain a C/N ratio:

  Bacteria - 5-6:1

  Fungi -10:1

5. References

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- Coyne, M.S. (1999). *Soil Microbiology: an Exploratory Approach*. Delmar Publishers, Albany, New York.

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Course Name	Agricultural Microbiology
Lesson 13	Nitrogen Cycle: Concepts and Role in Soils
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

Learning Objectives

1. Students can visually break down the N cycle step-by-step involving role of microbes.

2. Deeper understanding of the flow of various forms of Nitrogen and its related implications.

1. Introduction:

- Nitrogen is the basic constituent of life and is vitally important plant nutrient. Generally, the total N- content of soils varies from 0.02% to 0.44%.
- The Indian soils are generally poor in organic matter and consequently, have low N- content due to the tropical and subtropical climates.
- It exists in a number of oxidation states. Several of the redox reactions of N are carried out solely by microorganisms and the microbial involvement in the nitrogen cycle is of great importance.
- Thermodynamically N_2 gas is the most stable form of nitrogen.

1.1. Sources of Nitrogen in Soil:

- Parent material
- Plant residues and organic wastes
- Atmospheric nitrogen via biological nitrogen fixation
- Irrigation water
- Fertilizer

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1.2. Forms of N in soils

Nitrogen in soil exists in two major forms:

i) Organic N – amide form
ii) Inorganic N – Ammonium ion, nitrite ion, nitrate ion

- The organic N, particularly the hydrolysable form is slowly mineralised and is transformed to mineral N through aminization, ammonification and nitrification processes and becomes available to crops.
- The inorganic forms i.e., NH_4^+-N, NO_3^- -N, NO_2^- -N are very important for crop because plants take up N from the soil in inorganic form.
- The NO_2^- form is unstable and is usually present in soil in lesser extent.

1.3. Functions of Nitrogen:

- Essential constituent of proteins, amino acids, nucleic acids, nucleotides, enzyme, hormones, vitamins, alkaloids and phosphatides and an integral part of chlorophyll.
- It imparts vigorous vegetative growth and dark green colour to plants.
- It improves quality of leafy vegetables and fodders and also protein quality of the food grains.

2. Nitrogen transformation in Soil:

In the process of decomposition of plant and animal residues, the next most important element after carbon that undergoes microbial transformation is nitrogen.

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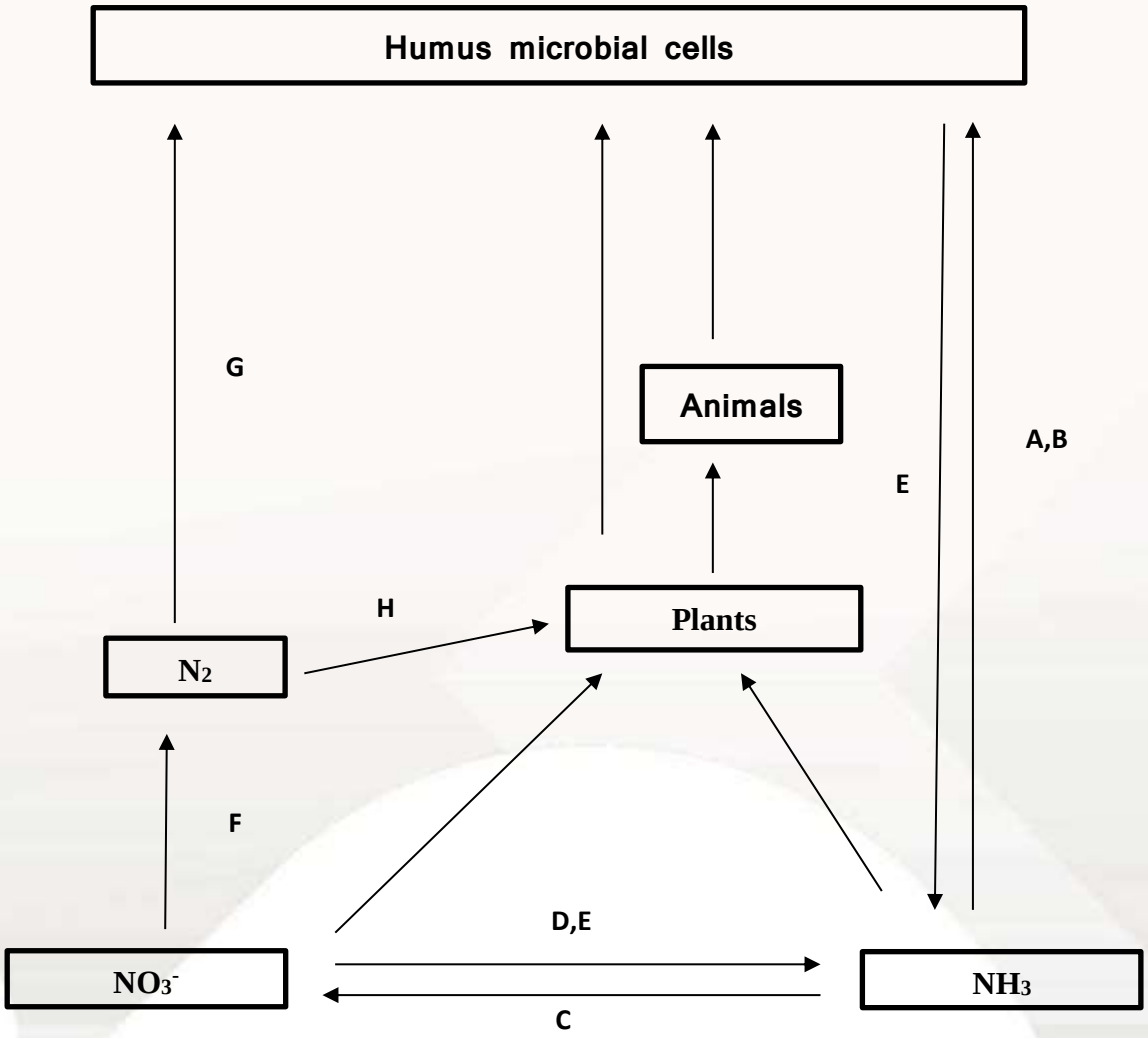

Figure 1. Basic framework of Nitrogen cycle; in this figure,

A- Ammonification ;

B- Mineralisation

C- Nitrification

D- Nitrate reduction

E- Immobilisation

F- Denitrification

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G- N_2 fixation (no symbiotic)

H- N_2 fixation (symbiotic)

- Such transformations are vital for making nitrogen available to plants and their growth. Only a very small part of atmospheric nitrogen is biologically fixed as proteins and other nitrogenous compounds in plants and microorganisms.

2.1. Ammonification:

- The conversion of nitrogen containing organic molecules (amines/ amino acids) into ammonia by microbial enzymes.

$R\text{-}NH_2 + H_2O \longrightarrow NH_3 + R\text{-}OH + Energy \longrightarrow 2NH_4^+ + CO_3^{2-}$

2.2. Mineralisation:

- Mineralisation is the process by which organic nitrogen gets converted into the inorganic ammonium or nitrate mediated by heterogeneous microorganisms. It covers the whole series of reactions carried out by microbes till the formation of nitrate.

2.3. Nitrification:

- The process of enzymatic oxidation of NH_4^+ to NO_3^- which is carried out by certain nitrifying microorganisms. Two groups of aerobic chemoautotrophs, *Nitrosamines* and *Nitrobacteria* are the main nitrifying organisms.
- Nitrification is a two- step oxidation process i.e., conversion: i) NH_4^+ to NO_2^-

ii) NO_2^- to NO_3^-

$$NH_4^+ + O_2 \longrightarrow NO_2^- + 2H^+ + H_2O + \text{energy}$$

$$NO_2^- + O_2 \longrightarrow NO_3^- + \text{energy}$$

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- First and second reaction is carried out by *Nitrosomonas* and *Nitrobacteria* group respectively

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2.4. Nitrate reduction (Volatilisation):

- It involves two enzyme-catalysed process that occur in roots or leaves depending on plant species.

Reduction reaction	Enzyme	Reaction site
I. $NO_3^- \longrightarrow NO_2^-$	Nitrate reductase	Cytoplasm
II. $NO_2^- \longrightarrow NH_3$	Nitrite reductase	Chloroplast

Volatilisation occurs when there is free NH_3 at soil surface. It generally occurs at alkaline pH.

2.5. Immobilisation:

- The process of conversion of inorganic NH_3 and NO_3^- into organic N is termed as immobilisation.
- During nitrogen immobilisation, microbes assimilate inorganic nitrogen for the synthesis of proteins and other nitrogen- containing organic compounds.

2.6. Denitrification:

- This is the microbial process in which nitrite and nitrate gets converted into molecular nitrogen or nitrous oxide, which goes into atmosphere.
- This process of denitrification is mediated by *Pseudomonas*, *Bacillus*, *Paracoccus*, *Thiobacillus denitrificans*, etc.
- The anaerobic condition of soil is conducive to the process of denitrification.

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2.7. A symbiotic N_2 fixation:

- Certain free- living bacteria like *Azotobacter*, *Clostridium*, *Beijerinckia*, *Enterobacter* fixes atmospheric N a symbiotically.
- However, the amount N fixed is low and is only 5-10 kg N/ha/year.

2.8. Symbiotic N_2 fixation:

- It is a biochemical process in which atmospheric N gets converted into reduced form in presence of bacteria.
- Genus *Rhizobium* is known to fix atmospheric nitrogen in legumes and makes nitrogen available to plant.

3. Factors affecting nitrification in soil:

- Soil pH: Nitrification takes place over a wide range in pH 5 to 9 and with an optimum around 8.5.
- Soil aeration: Adequate supply of oxygen that permit rapid gas diffusion are important for nitrification.
- Soil moisture: N transformation is highest at field capacity.
- Soil temperature: Very low, near freezing and increases rapidly upto 35ºC. Optimum soil temperature is 25 to 35ºC.

4. Nitrogen loss from Soil- Plant System:

- Leaching loss
- Ammonia volatilisation
- Denitrification loss
- Ammonium fixation by clay minerals
- Soil erosion and runoff
- Plant uptake

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Course Name	Agricultural Microbiology
Lesson 14	Phosphorus Cycle
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

Learning Objectives

1. To understand the transformation of phosphorus affecting its availability in soils.
2. To comprehend the role of microbes in mineralization and immobilization of phosphorus.

1. Introduction

- Phosphorus is second to Nitrogen as an inorganic nutrient needed by plants and microorganisms.
- It is an essential component of RNA, DNA, ATP and phospholipids. This element may be added to soil in the form of chemical fertilizers, or it may be incorporated as leaf litter, plant residues or animal remains.
- In cultivated soils it is present in abundance (i.e. 1100 kg/ha), but most of which is not available to plants, only 15 % of total soil phosphorus is in available form.
- Thus, phosphorus occupies a critical position both in plant growth and in the biology of soil.

1.1. Forms of phosphorus

- Phosphorus is present in the terrestrial environment in several forms and also in different pools.
- Major phosphorus pools are: - a). Absorbed (soluble), b). Organic Phosphorus c). Mineral Phosphorus.

Note: Absorbed P is the anion orthophosphate $PO4^{-3}$, $H_2PO_4^-$ and HPO_4^{-2}

- Much of the Organic Phosphorus in Soil is in unidentified forms.
- The most common identified form is inositol phosphate and it account for 15 to 30% of the total organic phosphorus in soil.
- Other identifiable organic forms of P in soil are nucleotide (2-5%) and phospholipids (1-2%).

There are more than 200 mineral forms of P in soil. For example,

$$M_{10}\,(PO_4)_6X_2$$

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Where M = Ca, Mg

X = Cl, F, OH^-, CO_3^{-2}

2. Availability of phosphorus in Indian soils

- The phosphorus nutrient is estimated to be in insufficient amounts in most of the Indian soils as available P. According to one of the compilations in 1994, based on about 9.6 million soil tests for available P in Indian soils, it is observed that 49.3% of areas covering different states and Union Territories are in the low category, 48.8% in the medium category and 1.9% have high P status.
- The limited high P available areas were only in Assam, Himachal Pradesh and Rajasthan.
- Therefore, application of phosphatic fertilizers is unavoidable in intensive farming system.

3. Phosphorus availability

- Under acidic conditions, P ions are present as H_2PO_4 but are subjected to fixation with hydroxides of Al and Fe at pH below 5.0.
- Near neutral pH, $HPO4^{2-}$ ions are usually present. But above pH 8.0, the PO_4^{3-} ions form Ca_3 $(PO4)_2$ and its availability is reduced drastically.
- The P cycle in soil involves the uptake of P by plants and its return to the soil in plant and animal residues.
- Although much is known on the subject, specific information is required on dynamic processes occurring within the cycle, such as conversion of the P of plant and animal residues to inorganic phosphate through microbial activity, chemical and biochemical changes that continuously take place in the various P compounds of the soil, and transformations that occur as P is absorbed and utilized by microorganisms and higher plants. A complete understanding of the P cycle is of particular importance in tropical soils, where most of the P occurs in organic forms.

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- Unique properties of the phosphorus cycle include the observation that phosphorus does not undergo any valence change in the cycle and that there is no gaseous component to the cycle. But P can exist in air either as phosphite (PO_3), phosphine (PH_3) and hydrogen phosphide (H_2P_4).

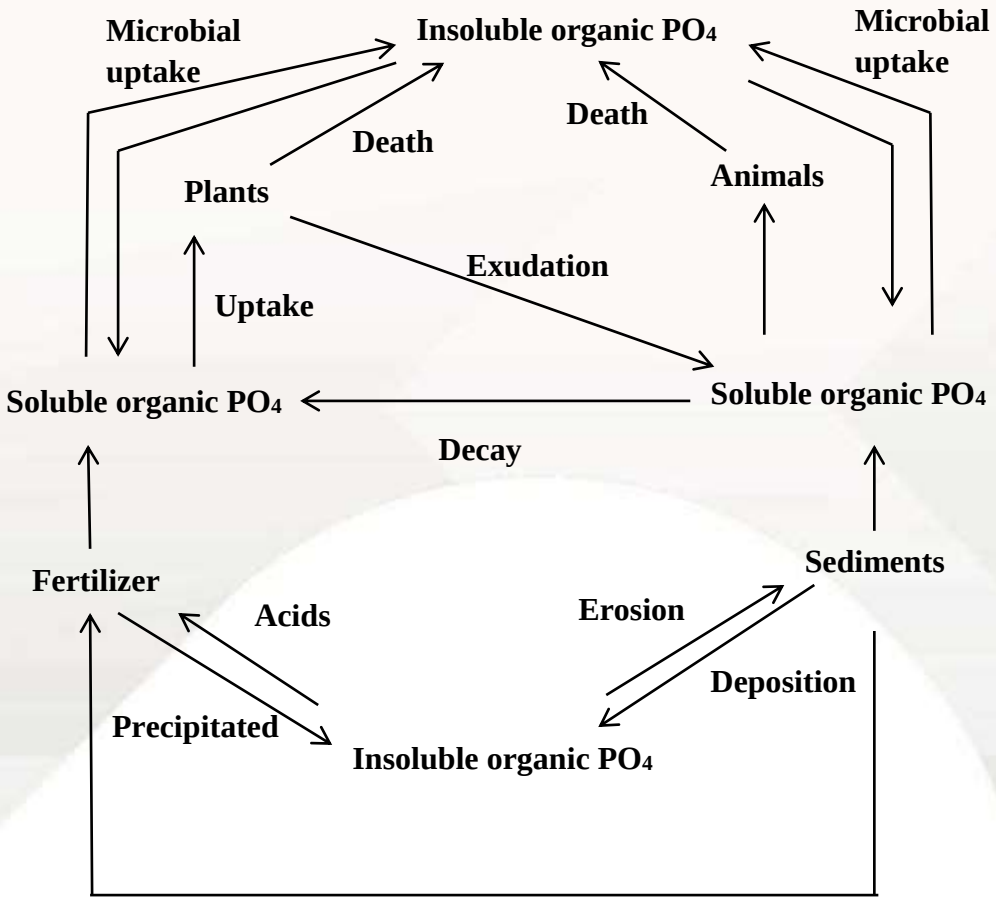

Figure 1. The terrestrial phosphorus cycle

4. Immobilization

- The concentration of P in soil solution is typically 0.1 to 1.0 ppm. Microbial concentration of Phosphorus are 10 times higher than they are in plants. The bulk of P in micro-organisms is in RNA (30-50%).
- At low P Concentration, micro- organisms accumulate P form inorganic or organic sources at the expense of plants.

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- Phosphates gets into the cells and is stored as polyphosphates. The end result is that bacteria can immobilized P and make it unavailable to plants.

5. Mineralization

- In this process, microorganisms convert the organic phosphorus to inorganic forms.
- Organic P which makes up 30-50% of the total P in soil, must be mineralized before it is available.
- Principal enzymes involved in P mineralization are phases and phosphatases. Although, phase are wide spread among organisms, they are limited by the small amount of phytin in soil.

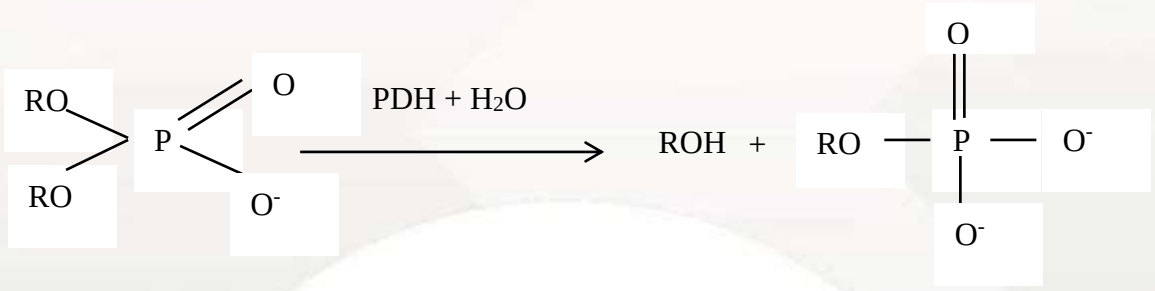

Figure 2. Mineralization of a phosphate diester

6. Chemical Fixation

- PO_4^{-3} fixation is the conversion of soil solution P to insoluble compounds by soil components, causing reduction in the amount that plant roots can absorb.
- Soil solution pH has significant effect on phosphate fixation. In neutral to alkaline soils (pH 7 and above), phosphates gets absorbed on calcium carbonate and are then participate as TCP and slowly to insoluble apatite's.
- In acid soil Fe and Al react with phosphate to form highly insoluble compounds.
- In neutral to alkaline soils

$$Ca(H_2PO_4)_2 + 2CaCO_3 \longrightarrow Ca_3(PO_4)_2 + 2CO_2 + 2H_2O$$

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- In acid soil:

$$Al^{+3} + H_2PO_4^- + 2H_2O \longrightarrow 2H^+ + Al(OH)_2H_2PO_4$$

(Alumino-hydroxy phosphate)

7. References

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Course Name	Agricultural Microbiology
Lesson 15	Sulphur Cycle in Soils
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

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Multiple Choice Questions:

1. Sulphur is the ---- most abundant element in nature?
   a) 6^{th}
   b) 8^{th}
   c) 9^{th}
   d) 10^{th}

2. In arid regions, Sulphur is present as:
   a) Pyrites
   b) Sphalerite
   c) Chalcopyrite's
   d) All of these

3. Sulphur is reduced to the …….group in (-SH) in plants
   a) Sulphoxide
   b) Sulphhydryl
   c) Sulphonyl
   d) None of these

4. Of the total Sulphur in soils, about ……. is in organic form.
   a) 5-10%
   b) 15-20%
   c) 30-50%
   d) 75-90%

5. Mineralization of Sulphur is a …….process
   a) Reduction
   b) Oxidation
   c) Electro-chemical
   d) Physico-chemical

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6. Immobilization of Sulphur is a ……..process
   a) Reduction
   b) Oxidation
   c) Electro-chemical
   d) Physico-chemical

7. When the C:N ratio of residues is <200:1, it will leads to
   a) Immobilization
   b) Mineralization
   c) Assimilation
   d) Dissimilation

8. When the C:N ratio of residues is >400:1, it will leads to
   a) Immobilization
   b) Mineralization
   c) Assimilation
   d) Dissimilation

9. pH of acid sulphate soils are around
   a) 2.0
   b) 4.0
   c) 6.0
   d) 8.0

10. Toxicity of hydrogen sulphides (H_2S) causes -------- disease
    a) Sheath Rot
    b) Blast
    c) Akiochi
    d) Brown Spot

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Answer

1. 1.10^{th}
2. *All of these*
3. Sulfhydryl
4. 75-90%
5. Oxidation
6. Reduction
7. Mineralization
8. Immobilization
9. 2.0
10. Akiochi

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Course Name	Agricultural Microbiology
Lesson 16	Biological Nitrogen Fixation: Implications in Agriculture
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

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Learning Objectives

1. To describe the importance of nitrogen fixation in agriculture.

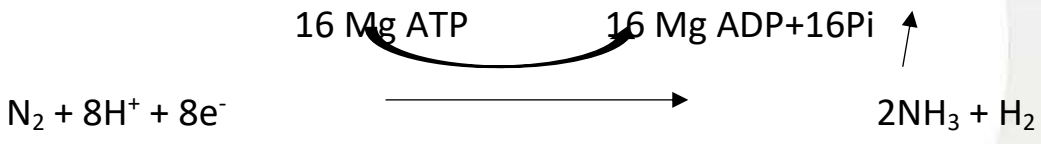
2. To develop an understanding of the various class of nitrogen fixation.

1. Introduction

- Biological nitrogen fixation is a biochemical process associated with plants.
- This process is carried out by different microbes like bacteria, actinomycetes, BGA, fungi and Yeast.
- Out of these bacteria is most important. Bacteria have the ability to fix the atmospheric Nitrogen in symbiosis with plants or as free-living organisms.
- Among these, symbiotic and non-symbiotic fixers of N are commonly used in agriculture. Free living BGA, can supply 30-40 kg N/ha, while *Azolla* a fern, in association with BGA can supply 30-40 kg N/ha.

2. Symbiotic Nitrogen fixation

- *Rhizobia* species are capable of having symbiotic relationship with leguminous crops of vital agricultural importance. They infect the plants roots and form nodules in them. In these nodules atmospheric N_2 reduced to ammonia with the help of nitrogenase enzyme.



- Most of the N fixed in nodule is utilized by the plants themselves. Out of that some nitrogen is released in the form of amines amino acids into the soil.

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- Upon the death of the microorganisms and incorporation of the roots, the nitrogen remaining in the nodules is added to the soil.
- The infection of plant root takes place when the C:N ratio is narrow. During the initial stages, the nodules absorb some N from soil. That's why a small amount of 15-20 kg N ha^{-1} is beneficial to stimulate the growth of seedlings. While excess application of nitrogen inhibits the activity of glutamine synthase and nitrogenase enzymes.
- The amount of N fixed ranges from 40 to 200 $kgha^{-1}$ and even more than that.
- There are different factors which influences the amount of nitrogen fixation includes, effectiveness of bacterial strain, available nitrogen in soil, applied N, application of Phosphorus, potassium and secondary nutrients and favourable soil environmental condition like temperature, moisture and ph.
- The mere presence of nodules on legume roots does not necessarily mean that they would fix nitrogen. The occurrence of red colour leghaemoglobin in the nodules indicates the presence of effective Nitrogen fixers. All legumes do not have nodules too (like rajma).

3. Biological nitrogen fixation by some legumes

Crops	**Nitrogen fixed in kg/ha/annum**
Mung bean	70
Soybean	105
Beans	58
Peas	48
Horse gram	40

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Groundnut	42
Cowpea	90
Lentil	130

Source: Fundamental of Soil Science (ISSS, New Delhi)

4. Non-symbiotic Nitrogen fixation

- Blue-green algae and Azolla: - Autotrophic BGA plays an important role in N_2 fixation in waterlogged rice culture.
- More than 125 species of BGA fix atmospheric nitrogen.
- Some well-known species are *Nostoc, Calothrix, and Anabaena etc.* They are aerobic and photosynthetic.
- The amount of biological nitrogen fixation varies from 14-15kgha^{-1}year^{-1}.
- In water bodies, a close association has been observed BGA, Anabaena and an aquatic fern, *Azolla pinnata*. The algae live in cavities in the leaves of fern.
- *Azolla* is used as green manure crop in the South East Asia under ideal condition as these association can meet 30kg N/ha to 50% of N requirements of rice.

5. Free living bacteria and other organisms

- Some microbes like bacteria, fungi, yeast and antinomy cetes living in soil and fix nitrogen.

Aerobes; *Azotobacter, Azospirillum, Beijerinckia, Enterobacter, Derxia*

Bacteria

Anaerobe; *Clostridium, Aerobacter, Methanobacterium, Rhodospirillum*

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- Note: The amount of nitrogen fixation by free living bacteria is comparatively low i.e. 5-10kg N/ha/year

6. Fixation by Tree and Shrubs

- In agro-forestry systems, both leguminous and non-leguminous trees and shrubs fix considerable nitrogen.
- For example, *Mimosa* and *Acacia* are the leguminous plants which fix N.
- Some non-leguminous plants which fix nitrogen are *Alnus*, *Myrica*, *Umptonia* and *Casuarina* etc.
- Phyllospheric fixation of N_2 carried out by *Frankia*, an actinomycetes, has also been reported in these plants.

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Course Name	Agricultural Microbiology
Lesson 17	Rhizosphere: Niche for microbes
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

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Learning Objectives

1. To develop an understanding about the various zones of rhizosphere.
2. To discuss the importance of rhizosphere effect and its causes.

1. Introduction

- The rhizosphere is the volume of soil under the vicinity or influence of the roots. It extends up to 2mm from the root and root hairs.
- Rhizosphere is the narrow region of the soil that is directly influenced by root secretion and associated micro-organisms are known as root microbiome.
- In 1904 the term rhizosphere coined by Hiltner to describe the plant-root interface, a word originating in past from the Greek word "*rhiza*", meaning root. Hiltner described the term rhizosphere as the area around a plant root that is inhabited by a unique population of microorganisms influencedby the chemicals released from plant roots.

2. Divisions of rhizosphere:

There are three zone of rhizosphere:

- The end rhizosphere : Endorhizosphere includes the portions of the cortex and endodermis in which microbes and cations can occupy the "free space" between cells (apo plastic space)
- The rhizoplane: It is the medial zone directly adjacent to the root including the epidermis and mucilage.
- Ectorhizosphere: It is the outermost zone which extends from the rhizoplane out into the bulk soil.

3. Why this is a critical zone for microbes

- Exudates such as organic acid, changes the chemical structure of rhizosphere in comparison with bulk soils.

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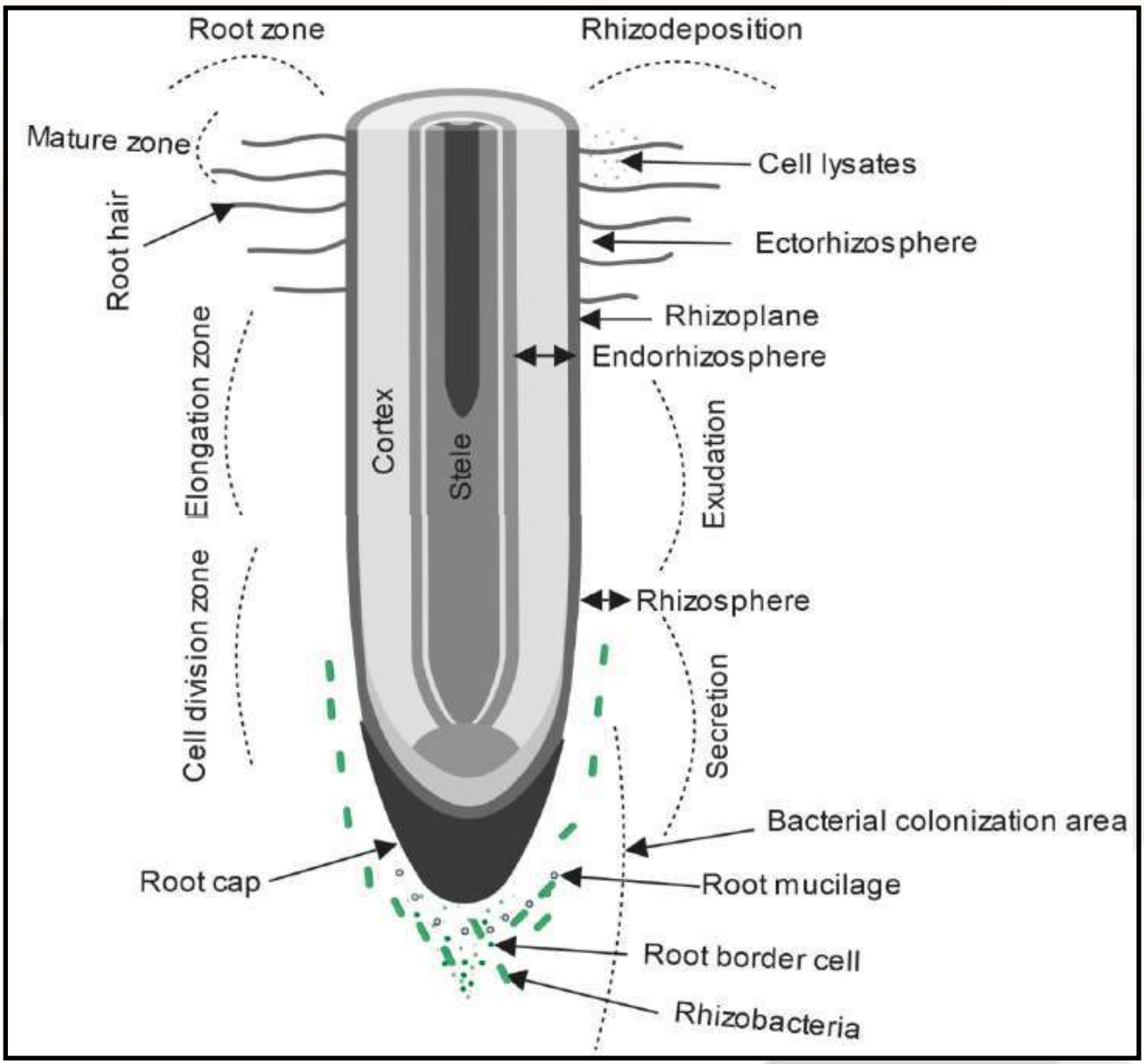
- Concentration of organic acid and saccharide, affect the ability of plant to obtain phosphorus, nitrogen, potassium and water through the root cap.
- Exudates come in the form of chemicals released into the rhizosphere by the cells in the roots and cell waste referred as rhizodeposition. This rhizodeposition come in various forms of organic carbon and nitrogen and it is given to the communities around the plant roots and dramatically affect the chemistry of rhizosphere.



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- Exopolysaccharides such as PGA affect the ability of roots to uptake water by maintaining the stability of rhizosphere and controlling the flow of water.

Figure 1. Types of different root zones

4. Root Derived Products:

- Newman (1985) examined a variety of plant species and estimated that roots can release anywhere from 10-50 mg C / g root produced or about 10-40% of their total photo-synthetically fixed carbon.
- The C releases in both organic (low molecular weight organic acids) and inorganic (e. g. HCO_3) forms. However, the organic forms are the most varied and biological process in the rhizosphere.
- The chemical compounds or root exudates which are secreted through roots in to the rhizosphere are known as rhizodeposition.
- Small molecules that are released from plant roots, which include sugars, amino acids, organic acids and amides.
- These molecules influence soil nutrient availability both directly and indirectly by stimulating the activities of certain microbial and fungal components of the soil biota.
- When plants begin to decline, the amount of organic carbon released as exudates increases. Mineral deficiencies, low amounts of soil air and severe wounding are major causes for the increase. Another way to say this is that an increase in exudates would be caused by over-pruning, construction injury, planting too deeply, over-watering, compaction and planting plants in soils that have a pH too high or too low for their optimal growth.

5. Types of root exudates:

- Secretions: Here the compound of low and high molecular weight which are released as result of metabolic process.

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- Lysates: these are the compounds released from autolysis of older epidermal cells.
- Mucilages: it is originating in root cap secreted by Golgi bodies of root cap cells
- Mucigels: gelatinous material at the surface of root grown in normal non-sterile soil.

Table 1. Compounds detected in root exudates

Classes of organic compounds detected in root exudates	**Exudate Compounds**
Sugars	Glucose, Fructose, Sucrose etc.
Amino Compounds	Asparagine, Glutamine, Aspartic acid, Lysine etc.
Organic acids	Tarataric, Oxalic, Citric, Malic etc.
Fatty acids and Sterols	Palmitic, Oleic, Cholesterol, Stigmasterol etc.
Growth factors	Biotin, Thiamine, Niacin, Choline etc.
Nucleotides, Enzymes	Flavonone, Adenine, Guanine, Amylase, Proteinase etc.
Miscellaneous	Auxins, Glycosides, Saponin, Zoospore attractants, bacterial stimulants and inhibitors etc.

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6. Rhizosphere effect:

- Rhizospheric effect is the ration of microbial population present in the rhizospheric soil and microbial population present in the non rhizospheric soil.
- When the ration is more than 1 it considered good, if it is equal to 1 it considered not good not bad and if its value is less than 1 it considered as inhibition for microbial growth.
- Plant by way of its rhizosphere creates subterranean habitat for microbe's stimulation of microbial growth due to root influence/ root rhizosphere influence is termed as rhizosphere effect and extent of stimulation is quantitatively expressed by the term R:S ratio.
- The R:S ratio is determined by the number of microorganisms or rate of biochemical process per gm of rhizosphere/ microbial organisms in a gram of bulk soil. Hence a value more than 1 signify stimulation otherwise inhibition.

Table 2. R:S ratio of various categories of microorganisms

Microbial number	**R:S ratio**
Algae	0.4
Protozoa	2.5
Fungi	12
Bacteria	24
Ammonifier	125
Denitrifier	1250

6.1. Factors responsible for rhizosphere effect

- Release of soluble organic compound by plant root.
- Debris derived from root cap cells and dying root hairs.
- Plant root lysis.
- Higher concentration of CO_2, lower concentration of O_2.

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- Low concentration of nutrient ions.
- Partial desiccation of soil due to absorption of roots.

7. Importance of rhizosphere

- Improve nutrient status- Members of the rhizosphere microbiome can significantly influence the nutrient status of plants. Well-known examples are the nitrogen-fixing rhizobia and the mycorrhizal fungi that facilitate phosphorus uptake.
- Supporting plant growth under biotic stress **-** The rhizosphere provides the frontline defence for plant roots against attack by soil borne pathogens.
- Nutrient cycling- Rhizospheric microbes are the core of cycling nutrients in soil considered important for plant growth.

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Course Name	Agricultural Microbiology
Lesson 18	Phyllospheric Microorganisms
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

Learning Objectives

1. To recognize plant parts as a habitat of microbes.

2. To develop an understanding of the factors affecting phyllospheric microorganisms.

1. Introduction

- The term phyllo sphere was coined by the Dutch microbiologist Ruinen J. in the year 1961.
- The phyllo sphere is a term used in microbiology to refer to the total above ground portions of plants as habitat for microorganisms.
- The phyllosphere is the aerial region of the plant colonized by microbes, its colonies are often called as epiphytes.
- Leaf surfaces can be densely populated by microorganisms. The most dominant group of microorganisms in the phyllosphere are bacteria, which reach a surprisingly dense population of on average 10^4–10^5 bacteria mm^{-2} of leaf surface or up to 10^8 bacteria g^{-1} leaf material.
- The dominant and useful microorganisms on the leaf surfaces in the forest vegetation Indonesia happened to be nitrogen fixing bacteria such as *Beijerinckia* and *Azotobacter* (As studied by Ruinen).
- In general, apart from nitrogen fixing bacteria like *Azotobacter*, other genera such as *Pseudomonas*, *Phytomonas, Erwinia, Sarcina* and other unidentified ones have been encountered on plant surface, especially on leaf surface.

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- The establishment of micro-flora on leaf surfaces is being aided by cuticle. Waxes and appendages help in providing anchorage to the microorganism's multiplication.
- Some of the fungi and actinomycetes recorded on plant surface are: *Cladosorium*, *Helminthosporium*, *Podospora*, *Uncinula*, *Bullera*, *Cryptococcus*, *Saccharomyces*, *Penicillium*, *Fusarium*, *Verticillium*, *Mucor*, *Aspergillus*, *Rhizopus*, *Actinomyces* and *Streptomyces*.

2. Division of phyllosphere

The phyllo sphere can be further subdivided into

A) Caulosphere (stems): Zone of stem colonized by microorganisms.

B) Phylloplane (leaves): Zone on leaves which colonized by the microorganisms.

C) Anthosphere (flowers): Zone on floral parts which colonized by the microorganisms.

D) Carposphere (fruits): Zone on fruits surface which colonized by the microorganisms.

3. The leaf surface as a habitat for microbial growth

- Environmental condition at the leaf surface exerts a tremendous influence on microbial population on the phyllo sphere, which in turn determine the interaction that can occur between the plants and microbes.

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- Growth on leaf surface frequently limited by both water and nutrient as well as exposure to high level of ultraviolet(UV) radiation, variation in physical and chemical landscape of the leaf itself and patchiness of nutrients on the leaf surface impose additional constrain for colonizing microorganisms.
- Most leaf associated microorganisms occur in areas that are somewhat protected from abiotic stress and areas that are closer interaction with the host plant.
- Denser population of bacteria are found in the grooves between the plant cell, at the base of trichrome, near the leaf vein and stomatal opening.
- Moisture availability is the key limitation of microbial growth on the leaf surface. The cuticle prevents moisture leaves and limits how much amount of water remain in the leaf surface. To overcome this challenge the bacteria may form aggregates and biofilm, produce extra cellular polymeric substances (EPS) which can resist desiccation.
- Microorganisms on the leaf surface are generally oligotrophy that can tolerate low nutrient condition as are microorganisms that interact with the host plant to obtain more nutrient.

Figure 1. The Phyllospheric Zone

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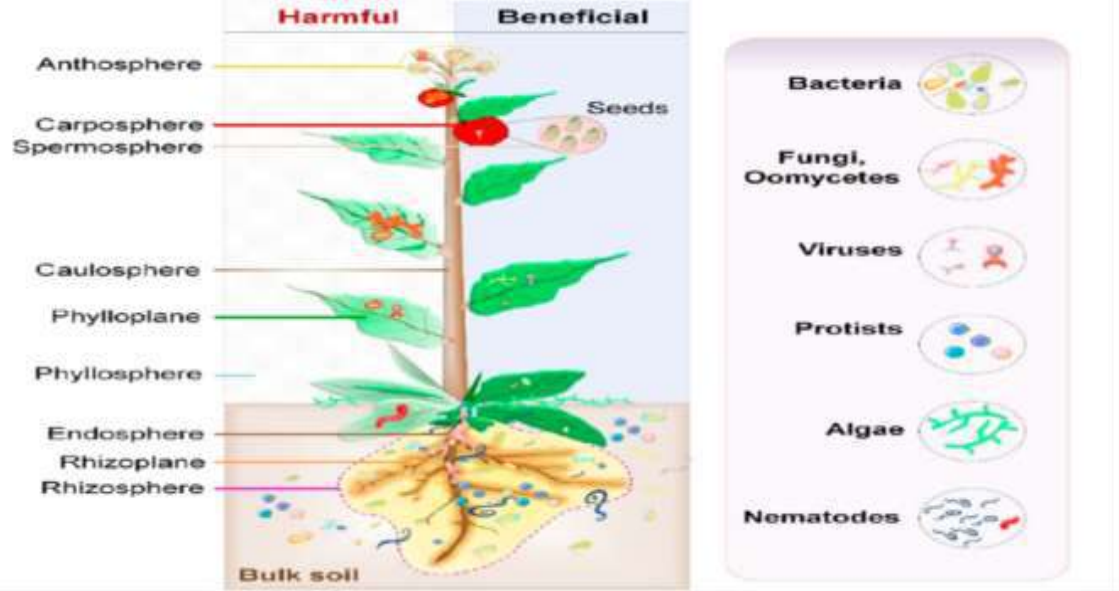
- Some plant metabolites can move to the leaf surface and supporting microbial growth.



- Phyllospheric microorganisms may obtain from plant produced amino acid or organic form of nitrogen that leach from the apoplast or by Nitrogen fixation.

4. Factors affecting growth of phyllospheric microorganisms

4.1. Light

- Since, plant parts directly receive sunlight on them and hence largely influenced by lights.
- There are bacteria that can easily harness light to produce chemical compounds that promotes growth of plants.
- Even, there is a role of UV radiation that has a role to play in the formation of secondary metabolites.

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4.2. Temperature

- The diurnal changes in temperature highly influence the phyllospheric region.
- Temperature affects the rate of various ongoing processes (Physical, Chemical and Biological) in the plant parts as well as in the residing microbes.
- Besides, temperature influences the heat and moisture in the leaf surface.

4.3. Plant species

- Growth of different plant species creates different microenvironment.
- Nutrient contents of plant parts can cause the variation in the microbes present.
- Plant age and developmental stage also affects the microbial composition.

4.4. Microbe-microbe interactions

- Phenomena like cooperation, parasitism, and competition.
- Each group of microorganisms play a unique and vital role in microbiomes, and their absence could cause a significant alteration in microbiome composition and functioning.

5. Some common phyllospheric micro-flora generally observed

- **Bacteria-***Enterobacter, Pseudomanoas, Bejerinckia,Azotobacter*

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- **Fungi-***Aspergillussp, Alternaria, Cladosporium,Cercospora, Candida, Fusarium, Verticillium, Colletotrichum, Penicillium*, etc.
- **Actinomycetes-***Actinomyces, Streptomyces*

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Course Name	Agricultural Microbiology
Lesson 19	Composting: Role of microbes
Content Creator Name	Rajiv Rakshit
University/College Name	Bihar Agricultural University, Bhagalpur
Course Reviewer Name	Shammi Kapoor
University/College Name	Punjab Agricultural University, Ludhiana

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Learning Objectives

1. To get acquainted with the process of composting.
2. To describe the role of microbes in each phase of composting.

1. Introduction

- Composting is a largely a biological process in which microorganisms of both types, aerobic and anaerobic decompose organic matter under medium to high temperature and lower the C:N ratio of the refuse.

- Composting is the controlled biological decomposition of organic matter into a stable, humus-like product called compost. It is essentially the same process as natural decomposition except that it is enhanced and accelerated by mixing organic waste with other ingredients to optimize microbial growth.

- Composting is a highly complex biodegradable process carried out by a diverse group of microbes. Different types of microbes are active at different phases of composting depending upon the availability of substrate, oxygen and moisture content.

- The biologically produced heat in this process sanitizes the material and minimizes the generation of odorous compounds and leachates.

- It led to a final product that is stable, free of pathogens and can be applied to land.

- Because of high organic matter content and due to relatively higher content of major nutrients compared to FYM, compost helps in maintenance of soil organic matter as maintains the physical and biological conditions of soils.

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1.1. Nature of compost

- **Rural Compost:** This utilizes weeds, crop stubble, crop residues, leaves, urine-soaked earth and litter from cattle shed
- **Town or Urban Compost:** Includes night soil, street and household katchara.

2. Method of Composting

2.1. Pit Method (Rural Compost)

- A pit is prepared where there is less chance of percolation of water.
- Residue is being added in a layer of about 30 cm thick.
- Over this, a layer of cattle dung and urine of 15 cm thickness is spread.
- Supply of extraneous source of nitrogen hasten the process.
- Under such anaerobic condition, high temperature does not develop and the decomposition is slow.
- Loss of ammonia is negligible.
- Advantage- No further attention and turning of the material required.

2.2. Heap Method (Rural Compost)

- This is aerobic in nature.
- Compost materials are heaped in layers
- Four to five layers are heaped one over another.

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- After 1.5-2 months, first turning is needed for mixing inside material with the outside ones.
- Generally, 2-3 turnings are needed for complete decomposition.
- Disadvantage is the two to three turnings.

2.3. Bangalore Method (Town Compost)

- Trench of suitable dimensions are needed.
- Site should be selected in the outskirts of the city.
- First, a layer of kathchara15 cm is spread on the bottom of trench.
- Over this, night soil is added (5 cm thick).
- Following this procedure, this dual layer is covered with katchara or loose earth.
- This layer will check foul smell, conserve moisture and avoid nitrogen losses.
- Nutrient content in compost: 1.4%N, 1.0% P and 1.4% K.
- Advantage: control foul smell, kills pathogenic microbes, avoid nitrogen losses.

3. Phases of composting

- During the first phase a diverse population of mesophilic bacteria and fungi proliferates, degrading primarily the readily available nutrients and thereby raising the temperature to about 45 °C. At this point their activities cease, the vegetative cells and hyphae die and eventually lyse, and only heat resistant spores survive.

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- Second phase is characterized by the development of a thermophilic microbial population comprising some bacterial species, actinomycetes and fungi. The temperature optimum of these microorganisms between 50 and 65 °C, their activities terminate at 70–80 °C.
- The third phase can be regarded as a stationary period without significant changes of temperature because microbial heat production and heat dissipation balance each other. The microbial population continues to consist of thermophilic bacteria, actinomycetes, and fungi.
- The fourth phase is characterized by a gradual temperature decline; it is best described as the maturation phase of the composting process. Mesophilic microorganisms having survived the high temperature phase or invading the cooling down material from the outside succeed the thermophilic ones and extend the degradation process as far as it is intended.

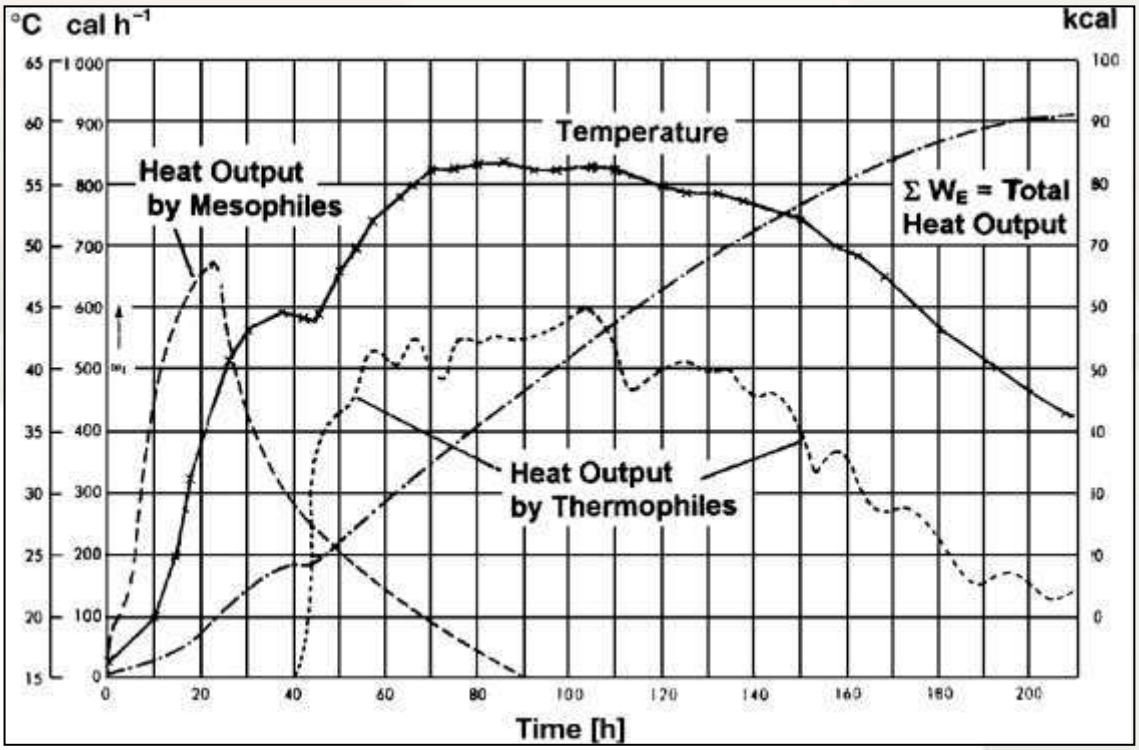

Figure 1. Temperature course during composting (From Popel, 1971)

4. Microorganisms involves during composting

Mesophilic phase:

- **Bacteria**-*Pseudomonas sp., Bacillus sp., Cellumonas sp.,*
- **Fungi**-*Fusarium, Trichoderma, Aspergillus, Mucor, Helminthosporium*

Thermophilic phase:

- **Bacteria**-*Bacillus sp., Streptothermophillus*
- **Fungi**-*Humicolla, Absidiachetonium*
- ***Actinomycetes***-*Micro-monosperma, Nocardia, Streptomyces, Termonospora, Thermopolyspora*

Approximate numbers of microorganisms during different phases of composting

Organism	**Numberg^{-1}substrate**
Bacteria in mesophilic stage	10^9-10^{13}
Bacteria in thermophilic stage	10^8-10^{12}
Actinomycetes, thermophilic stage	10^7-10^9
Actinomycetes, mesophilic stage	10^8-10^{12}
Fungi, average value	10^5-10^8

Source: Miller, 1993

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5. Factors affecting composting

5.1. C: N ratio

- An initial C: N ratio of 20:1 to 40:1 is recommended for rapid composting.
- Higher C: N ratios work more efficiently, but require large additions of a carbon source.
- If carbon is presenting excessive amounts relative to nitrogen so that theC:N ratio is above the optimal range, the composting process slows. In this case nitrogen availability is the limiting factor.

5.2. Oxygen

- Oxygen is necessary for the survival of aerobic microorganisms.
- If sufficient oxygen is not provided to sustain aerobic micro-organisms, anaerobic microorganisms begin to dominate the compost pile, slow the composting process.
- A minimum oxygen concentration of 5 percent is required to maintain aerobic conditions.

5.3. Water

- Water is another essential component for the survival of composting micro-organisms.
- The micro-organisms require an aqueous environment in which to move and transport nutrients.
- The ideal moisture content for composting must be a compromise between achieving adequate moisture for the micro-organisms to function and adequate oxygen flow to maintain aerobic conditions.

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- The moisture content for composting is generally recommended to be in the range of 40 to 65 percent. Below 15 percent moisture, microbial activity ceases altogether.

5.4. PH

- The pH levels of the raw material of the compost mix do not significantly impact the composting process because different micro-organisms thrive at different pH levels.
- The ideal range for microbial activity is between 6.5 and 8.0. Composting continues at extremes, such as 5 and 9, but the process slows.
- By the end of the composting process, the pH generally stabilizes between 7.5 and 8.0, regardless of the beginning ph.

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