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Agricultural Microbiology (ASC 121)

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Contents

INTRODUCTION

Microbiology is the study of living organisms of microscopic size, which include bacteria, fungi, algae and protozoa and the infectious agents at the borderline of life that are called viruses. It is concerned with their form, structure, reproduction, physiology, metabolism and classification. It includes the study of their distribution in nature, their relationship to each other and other living organisms, their effects on human beings and on other animals and plants, their abilities to make physical and chemical changes in our environment and their reactions to physical and chemical agents.

Microorganisms are closely associated with the health and welfare of human beings; some microorganisms are beneficial and others are detrimental. For example, microorganisms are involved in the making of yogurt, cheese, and wine; in the production of penicillin, interferon, and alcohol; and in the production of domestic and industrial wastes.

Most **microorganisms** are unicellular in which all the life processes are performed by a single cell. All living cells contain protoplasm which is a colloidal organic complex consisting of largely proteins, lipids and nucleic acids. Organisms are composed of many cells that are arranged in tissues and organs to perform specific functions. Regardless of the complexity of an organism, the cell is the basic structural unit of life. All living cells are fundamentally similar. Microorganisms occur everywhere in nature – In air, oceans, mountain tops etc. as the conditions for the growth and survival of the microorganisms are similar to those of human beings, they are in the air we breathe, and the food we eat. They are on the surface of our bodies, in our mouths, noses, alimentary tracts etc. Fortunately, most microorganisms are harmless to human beings and we have means of resisting invasionby those that are potentially harmful. Some microorganisms are beneficial and some are detrimental. Microbes are involved in making of cheese and wine, in the production of penicillin, interferon and alcohol, in the processing of domestic and industrial wastes. They can cause disease, spoilage of food; deteriorate materials like iron pipes, glass lenses and wood pilings.

History of Microbiology

Late Selman A Waksman –Microbiologist observed that there is no field of human endeavour whether it may be an industry or agriculture or in the preparation of food or in connection with problems of shelter or clothing or in the conservation of human or animal health and combating of disease where the microbe does not play an important and often dominant role. He discovered antibiotic Streptomycin produced by soil bacterium for which

he was awarded Noble Prize in 1952. The existence of microbial world was unknown until the inventions of microscopes, which were invented at the beginning of 17th century. The discoverer of the microbial world was a Dutch merchant Antony von Leeuwenhoek (1632-1723) with his microscope. His microscopes were able to give clear images at magnifications from about 50 to 300 diameters. Leeuwenhoek's place in the scientific history depends on the range and skill of his microscopic observations. He studied almost every conceivable object that could be looked through a microscope. He described the microbial world he observed as *'animal cules'* or 'little animals' . All the main kinds of unicellular organisms that we know today – protozoa, fungi, algae, & bacteria were first described by Leeuwenhoek. He was first to describe the Spermatozoa, RBC, free living as well as parasite protozoa & the bacteria which he called animal cules (small animals). He communicated all his observations to the British Royal Society in a series of letters. Leeuwenhoek's discovery of the animal cules & other microbes revealed the presence of a hitherto unknown world – the microbial world. However, the development of study of microorganisms into science 'Microbiology' has been delayed till late 19th century. The principal reasons for this long delay seems to have been technical ones.

After Leeuwenhoek's discovery of microorganisms, the origin of microbes became the subject of discussion. Some Scientists believed that animalcules were formed spontaneously from non-living materials, whereas others (including Leeuwenhoek) believed that they were from seeds or germs of these animalcules, which were always present in the air.

Spontaneous Generation:

The belief in the spontaneous formation of living beings from non-living matter is known as Doctrine of spontaneous generation (SG). This controversy existed for a long time. It became difficult to disprove this doctrine, because of lack of experimental proof. Later **Francesco Redi** in 1665 performed experiments and showed that maggots that develop in putrefying meat are the larval stages of flies and will never develop in putrefying meat if it is protected from flies laying eggs. He was the first to disprove SG of animals. **Lazzaro Spallanzani** (1729-99) was the first to provide evidence that microorganisms do not develop spontaneously. He boiled beef broth for an hour and then sealed the flasks. No microbes appeared following incubation. **John Needham** (1713-81) insisted that air was essential for SG of microbes. By sealing the flasks, the air had been excluded. This argument was answered after 60 – 70 years independently by two other scientists. **Franz Schulze** (1815-73) passed air through strong acid solutions into boiled infusions. **H.Schroder and T.Von Dusch**

(About 1850) passed air through cotton into flasks containing heated broth. Thus the microbes were filtered out of the cotton fibers and no microbial growth. Basic technique of plugging bacterial culture tubes with cotton stoppers was initiated. **Archimede Pouche** (1859) published an extensive report favoring SG. **Louis Pasteur** (1822-1895) the immortal French scientist, performed various experiments to disprove S.G. He developed a flask with a long, narrow gooseneck opening through which untreated and unfiltered air could pass in or out, but the germs settled in gooseneck. As germ free air entered the flask no microbes appeared in the infusion.In 1862, Louis Pasteur conducted experiments to disprove the theory of Spontaneous Generation. He prepared flasks, with long, narrow, goose-neck openings heated the nutrient broth in the flask and thus the air carrying the germs were allowed to settle in the goose-neck. When the flasks were cooled, the air entering through the gooseneck retained the germs, and under these conditions the broth remained clear. He also showed by further studies that 'used' cotton filters, when examined under the microscope, revealed the presence of microscopic organisms. **John Tyndall** (1820-1923) proved that dust carried the germs. He showed that sterile infusions placed in a dust free chamber could remain sterile indefinitely even if kept exposed to air. During his experimentation he concluded that bacteria have phases one relatively thermolabile (growing phase destroyed by boiling for 5 min.) and onethermo resistant (bacterial spores cannot be destroyed even by boiling for 5 ½ hours). He developed a method of sterilization by discontinuous heating, later called ***Tyndallization***,which could be used to kill all bacteria in infusions. Allowed the infusion to stand for a certain period, before applying heat to permit the germination of spores with a consequent loss of their heat resistance. Then boiled to kill bacteria. He found that discontinuous boiling for 1 min on 5 successive occasions would make the infusion sterile whereas continuous boiling for 1 hr. would not. Pasteur and Tyndall's experiments finally disproved the Doctrine of Spontaneous generation (S.G.).

MICROBIAL WORLD: PROKARYOTIC AND EUKARYOTIC MICROBES

DIFFERENT MICROBIAL GROUPS

The major groups of protists are briefly described below.

A) Procaryotic Protists

1. **Bacteria:** Unicellular, procaryotic, cell multiplication is usually by binary fission. Practical significance: Cause diseases, natural cycling- soil fertility, spoil food, make food.etc.

2. **Algae:** This prokaryotic group of primitive organisms are unicellular. Others are aggregations of similar cells with little or no differentiation in structure or function. Some algae such as large brown algae have a complex structure with cell types specialized for particular functions. Regardless of size or complexity, all algal cells contain chlorophyll and are capable of photosynthesis. Found in aquatic environments or in damp soil. Cyanobacteria (Blue Green Algae) is also a prokaryotic protist. Blue green algae are diazotrophs and having the capacity to build up soil organic matter level by virtue of deposition of appreciable amount of photosynthates in the soil, help to enhance water holding capacity of the soil, acts as soil conditioner, helps to energize the phosphate solubilizers and capable of producing phyto hormones

B) Eucaryotic Protists

1) **Fungi:** Eucaryotic lower plants devoid of chlorophyll. They are usually multicellular but are not differentiated into roots, stems and leaves. They range in size and shape from single celled microscopic yeast to giant multicellular mushrooms and puff balls. True fungi are composed of filaments and masses of cells, which make up the body of the organism called mycelium. They reproduce by fission by budding or by spores –molds, mildews, yeasts and rusts belong to this group. Yeasts are only unicellular fungi and molds are multicellular in nature.

2) **Protozoa:** Unicellular, Eukaryotic. Differentiation based on their morphological, nutritional and physiological characteristics. Best known protozoa are few that cause disease in human beings and animals.

BACTERIA: CELL STRUCTURE, CHEMOAUTOTROPHY, PHOTO AUTOTROPHY, GROWTH

Bacterial Structure:

Until the eighteenth century all the living organisms were grouped into two kingdoms, plant and animal. After the discovery of the microbial world, it is evident that some organisms are predominantly plant like, some are animal like and some others share the characteristics common to both plants and animals. Since there are organisms that do not fall into either plant or animal kingdom, it was proposed that new kingdom be established to include those organisms, which typically are neither plants nor animals. E.H. Haeckel in 1866 proposed a third kingdom 'Protista' to include the microorganisms those are typically neither plants nor animals. Bacteria, algae, fungi and protozoa are included in Protists (Viruses are not cellular organisms and hence not classified as protists). Bacteria are referred to as lower protists, whereas the fungi, algae and protozoa are called higher protists.

Procaryotic and Eucaryotic cells

With the aid of electron microscopy in 1940's it was discovered that in some cells, for example typical bacteria, the nuclear substance was not enclosed by a nuclear membrane. In other cells such as typical fungi, algae, and protozoa the nucleus wasenclosed by a membrane. This discovery led to the distinction of microbes into two groups *viz*., prokaryotes (incipient nucleus) and eukaryotes (true nucleus). Bacteria are prokaryotic microorganisms and are called as prokaryotes. Algae, fungi and protozoa are eukaryotic microorganisms and are referred as eukaryotes (plant and animal cells are alsoeukaryotic). The eukaryotic cells are characterized by the presence of multiplicity of unit membrane systems, which are structurally and topographically distinct from cytoplasmic membrane. These membrane systems enable the segregation of different eukaryotic cytoplasmic functions into specialized organelles. The major differences between procaryotic and eucaryotic cells are detailed below.

Morphological types of bacteria:

Knowledge of the morphology and internal structure of bacteria will be useful in classifying the bacteria and also to understand various physiological processes that are taking place in bacteria.

Size:

0.5 to 1.0 µm in diameter, surface area/ volume ratio is exceedingly high favouring unusually high rate of growth and metabolism of bacteria. No circulatory mechanism is required to distribute the nutrients that are taken in, due to this high surface to volume ratio.

Shape and arrangement:

The shape of bacterial cell is governed by rigid cell wall. They may be spherical (Coccus – Cocci), straight rods (Bacillus – Bacilli), or rods that are helically curved (Spirillum – Spirilli) or they may be pleomorphic (exhibit a variety of shapes). coccus bacillus spirillum monstrous microbe Epulopiscium Budding & Appendaged bacteria. The Cocci are further grouped into Diplococci, Streptococci, Tetracocci and Staphylococci based on the ncharacteristic arrangement of the cells. Bacilli are mostly singular or in pairs (Diplococci). But some species may be Streptobacilli (Ex: *Bacillus subtilis*) or trichomes (Ex: *Beggiatoa*) or may have palisade arrangement (*Corynebacterium diphtheria*). Some other bacilli may form long, branched multinucleated filaments called hyphae, which collectively form mycelium (Ex: *Streptomyces*) Mono, diplo, strepto, tetra, staphylococcus. The bacteria with less than one complete twist or turn have 'vibroid' shape, whereas those with one or more complete turns have a helical shape. Many other shapes also occur in addition to the above common shapes. The cell wall is common to all bacteria. The structures that are present external and internal to the cell wall are not common to all bacteria. Some characters are more specific to certain species.

Typical bacterial cell structure and functions of different parts of bacterial cells external structures:

Flagella (flagellum) and motility:

Bacterial flagella are hair like helical appendages that protrude through the cell wall and are responsible for swimming motility. It grows at the tip unlike hair, which grows at the bottom.

A flagellum is composed of 3 parts

a). Basal body associated with cytoplasmic membrane and cell wall

b). a short hook and c). A helical filament which is usually several times longer than the bacterial cell. The hook and filament are made up of protein whereas the composition of basal body is not known. The protein of the filament is known as flagellin.

Flagellar arrangement may be a). Monotrichous – a single polar flagellum b). Lophotrichous – a cluster of polar flagella c). Amphitrichous – flagella either single or clusters, at both cell poles and d). Peritrichous –surrounded by lateral flagella. Bacteria propel themselves by rotating their helical flagella. Without external flagella some helical bacteria (spirochetes) exhibit swimming motility by means of Endoflagella (flagella like structures beneath the outer cell envelope). Some bacteria ex: myxobacteria exhibit gliding motility only when they are in contact with solid surface. Most motile bacteria are able to change their movement in

response to environmental stimuli. These are called tactic movements. Bacterial chemotaxis is the movement of bacteria toward or away from the chemical compounds. Swimming of bacteria towards a chemical is known as positive chemotaxis, swimming away is negative chemotaxis. Phototrophic bacteria exhibit positive phototaxis toward increasing light intensities and are repelled by decreasing light intensities.

Pili (Fimbriae):

They are hollow non-helical filamentous appendages that are thinner, shorter and more numerous than flagella. Do not function in motility. Different types of pili have different functions. F – pilus (Sex pilus) serves as the port of entry of genetic material during bacterial mating. Some pili play major role in human infection.

Capsule:

Many bacteria synthesize organic exopolymers that form an envelope outside the cell wall. If this layer can be seen by light microscopy using special staining methods it is called a capsule. It is termed a microcapsule if it is too thin to be seen by light microscopy. The material is called "Slime" if the layer is abundant and many cells are embedded in a common matrix. Most pathogenic bacteria produce either capsule or slime. The functions of the capsule depend on bacterial species (1) they may block attachment of bacteriophages (2) they may be antiphagocytic (3) they may provide protection against temporary drying by binding water molecules (4) they may promote attachment of bacteria to surfaces.

Sheaths:

Sheath is a hollow tube formed in some species of bacteria to enclose chains or trichomes of bacterial cells. Sheath is commonly found in the species from fresh water and, marine environments.

Cell wall composition:

In bacteria the cell wall is very rigid and gives the shape to the cell. Most of the bacteria retain their original cells even after subjected to very high pressure or severe physical conditions. It accounts for 10-40% of dry weight of the cell. Cell walls can be broken by sonic or ultrasonic treatment or by subjecting the cells to extremely high pressure and subsequent sudden release of pressure. Cell wall composition of eubacteria is different from that of archaebacteria (bacteria are broadly distinguished into eubacteria and archaebacteria based on their ancestral relationships i.e., on evolution and genetic relatedness). Eubacteria cell wall is made up of peptidoglycan (murein and insoluble, porous cross linked polymer of enormous strength and rigidity. Peptidoglycan is basically a polymer of Nacetylglucosamine,

N- acetylmuramic acid, L- alanine, D-alanine, D-glutamate and a diamino acid. The peptidoglycan is present only in prokaryotes. The cell walls of archaeobacteria are generally made up of proteins, glycoproteins or polysaccharides. Gram positive and Gram negative type of bacteria are present in both eubacteria and archaebacteria. Gram staining is one of the most important and widely used differential staining introduced by Christian Gram in 1884. Bacteria stained by Gram's staining method fall into two groups –Gram positive, (which appear deep violet in color) and Gram negative (which appear red in color). Gram staining is generally not applicable to other microorganisms. However, yeasts consistently stain gram positive.

Differences in the cell wall of Gram positive and Gram negative eubacteria

Sl no.	Character	Gram Positive	Gram Negative
01	Thickness	Thicker wall (20-25 nm)	Thinner (10-15 nm)
02	Layers	A single thick layer	Two layers (a pepditoglycan layer and outer membrane)
03	Peptidoglycan	Account for 50% dry weight of cell wall	Only about 10% of cell wall
04	Other constituents	Polysaccharides and teichoic acids	Outer membrane is rich in phospholipids, proteins or lipopolysaccharides. Peptidoglycan layer is linked to outer membrane by Braun's lipoprotein.
05	Susceptibility to a. Penicillin b. Mechanical disintegration	 More susceptible Less susceptible	 Less susceptible More susceptible

Structures internal to cell wall

Cytoplasmic membrane:

This is about 7.5 ηm thick and is immediately beneath the cell wall. This is primarily composed of phospholipids (20-30%) and proteins (60-70%). This membrane contains various enzymes involved in respiratory metabolism and in the synthesis of capsular and cell wall components. It is the site of generation of proton motive force, which drives ATP synthesis, certain nutrient transport systems and flagellar motility. Damage to this membrane may result in the death of the cell.

Protoplast and Sphaeroplast:

A protoplast is that portion of a bacterial cell consisting of the cytoplasmic membrane and the cell material bound by it. This can be prepared from Gram positive bacteria by treating the

cells with an enzyme such as lysozyme, which selectively dissolves the cell wall or by culturing the bacteria in the presence of an antibiotic such as penicillin. Sphaeroplast is a protoplast surrounded by the outer membrane of cell wall. In gram-negative bacteria only peptidoglycan layer can be removed but outer membrane is still intact surrounding the protoplast.

Mesosomes:

In many bacteria, especially Gram-positive bacteria, the cytoplasmic membrane appears to be infolded at more than one point. Such infoldings are called mesosomes. Mesosomes are thought to be involved in DNA replication, cell division and export of extra cellular enzymes.

Cytoplasm:

The major cytoplasmic contents of bacterial cell include the nucleus, (without a membrane), ribosomes, proteins and other water soluble components and reserve materials. In most bacteria extra chromosomal DNA (Plasmid DNA) is also present.

Bacterial Chromosome:

The bacterial nucleus is not enclosed in a defined membranous structure. The nuclear material is generally confined to the center of the cell. It consists of single circular double stranded DNA molecule in which all the genes are linked. This nuclear material is generally designated as nucleoid, or chromatin body or nuclear equivalent.

Ribosomes:

Ribosomes are 70 S type consisting of 50 S and 30 S sub-units. Some ribosomes are free in the cytoplasm and some are attached to inner surface of the cytoplasmic membrane.

Volutin granules (reserve source of phosphate), poly-β-hydroxybutyrate (PHB) and glycogen (both serving as source of carbon and energy) are some of the granules present in the cytoplasm of some bacteria. Gas vesicles are present in bacteria that grow in aquatic habitat.

Spores:

Spore is a metabolically dormant form, which under appropriate conditions can undergo germination and grow out to form a vegetative cell. Spores produced within the cell are called endospores and the spores produced external to cell are called exospores. Endospores are thick walled, highly refractile bodies that are produced (one per cell) *by Bacillus, Clostridium, Sporosarcina* and few other genera. They are generally formed at the end of the active growth or during stationary phase. They are extremely resistant to desiccation, staining, disinfecting chemicals, radiation and heat. Exospores are formed external to the vegetative cell by budding at one end of the cell in the methane oxidizing genus *Methylosinus*. They are desiccation and heat resistant.

Conidiospores and Sporangiospores:

The bacteria, actinomycetes form branching hyphae. From the tips of these hyphae spores develop singly or in chains. If the spores are contained in an enclosing sac (sporangium), they are termed SPORANGIOSPORES, if not they are called CONIDIOSPORES. These spores can survive long periods of drying but they do not have high heat resistance.

Cysts:

Cysts resemble endospores in some ways, but their structure and chemical composition are different. Cysts are thick walled, desiccation resistant, dormant forms that, developed by differentiation of vegetative cells. *Azotobacter* and some other genera produce cysts.

Bacterial growth

The bacteria take up nutrients from their environment, which are converted into new cell substances like RNA, DNA, proteins, enzymes and other macromolecules. Growth is the orderly increase in all of the components of an organism. Thus, the increase in size that results when cell takes up water or deposits lipid is not true growth. Cell multiplication is a consequence of growth; in unicellular organisms, multiplication leads to an increase in the number of individuals making up a population or a culture.

Cell division and reproduction in bacteria:

Multiplication of bacteria takes place by one of the following methods – Transverse binary fission, Budding, Fragmentation, Conidiospores or Sporangiospores.

Transverse binary fission is the most common and important in the growth cycle of bacterial population, which is an asexual reproductive process. Infrequently, in some species, binary fission may be preceded by mating or conjugation of cells.

Fragmntation: Some bacteria produce extensive filamentous growth, which is followed by the fragmentation of these filaments into small bacillary or coccoid cells, each of which give rise to new growth. Eg. *Nocardia* species.

Spore Production: Some genera of bacteria produce reproductive spores called conidiospores or sporangiospores at the tip of filamentous growth, each of these spores give rise to a new organism. Eg : *Streptomyces*

Budding: A few bacteria also reproduce by a process known as budding where in the parent cell remains intact while a new cell buds off which again grows into a new organism.

Eg: *Rhodopseudomonas, Hyphomicrobium*

Generation time:

In unicellular microorganisms, growth usually, involves an increase in cell number. A single cell continually increases in size until it is approximately double of its original size; then cell

division occurs, resulting in the formation of two cells. During this cell division cycle all the structural components of the cell will double. The formation of two cells from one parent cell is called a 'generation time'. The generation time is thus the time required for the cell number to double. Because of this the generation time is also called as 'doubling time'. It is to be noted that during a single generation, both the cell number and the cell mass are doubled. Generation time vary widely among microorganisms anywhere between minutes to days, 20 minutes in *E. coli* to 33 hours in *Treponema.* The number of generations per hour is usually determined by plotting cell number against time on a semi logarithmic scale and reading off directly the time required for the number to double. Alternatively, the generation time can be calculated directly solving the equation for n = number of generations = log N- log N (o)/ log 2.

Generation time g = t/n.

If we start with a single bacterium the increase in population by geometric progression 1- 2- 2^2 -2^3 -2^4 – 2^n, where n is total number of generations.

Total population 'N' is expressed by N1 = lx2n at the end of given time period.

Total population at zero time is = N_0 Total population at end time is = N (f)

Population in given culture at time't' is N (f) = N0 X 2^n (or) initial population X 2^n .

log 10N(f) = log 10N (0) + log 102(n) (or) log 10N(f) = log 10N(0) +n log 10 2 (or)

n= log 10N(f)-log10N(0) log102 n = log 10 N(f) - log 10 N(o) ÷ 0.3010 =3.3 (log 10 N(o) - log 10 N(o)

For example, if an inoculum of 10 4 cells grows exponentially to 1 X 107 cells in 8 hours'time.

n= log 107 – log 104 ÷ 0.3010 = 7 - 4 ÷ 0.3 =10 generations.

If n generations are taken place in 5 hours' time, the growth rate was 10 / 5 = 2.0generations / hour and the generation time was=t/n= 5 X60min/10=30min or 0.5 hour. 1/g=1/0.5=2 generations per hour.

Growth of fungi is different from other unicellular organisms that divide either by binary fission or by budding. In filamentous fungi growth occurs only at the tips of filament and therefore is not exponential. Moreover, extensive branching occurs in fungi and new ends are generated at which growth occurs. Thus, fungi growth is difficult to describe in simple mathematical terms.

Growth rate:

Growth rate is the change in cell number or mass per unit time. It is expressed as 'R' which is the reciprocal of generation time 'g'. It can be defined as the slope of the

line when log of cells versus time is plotted (R = 1/g). Microbes generally respond linearly to a limiting nutrient concentration in the medium, which forms the principle for microbiological assays.

Growth yield

Balanced growth is a condition where all biochemical constituents are being synthesized at the same relative rates. Growth yield is the mass of cells produced per unit of a limiting nutrient concentration. It is denoted by Y = X-X0/C, where X0 = mass of initial population immediately after inoculation, X = mass of final population after cells enter stationary phase, C = concentration of the limiting chemical constituent in the medium. This is the basis used in microbiological assays of various vitamins and amino acids by auxotrophic mutants of bacteria.

Growth cycle of bacteria

When a liquid medium is inoculated with unicellular bacteria or yeasts or other budding organisms, the population undergoes a characteristic sequence of events during the increase in cell number. When the number of cell /ml is determined periodically and plotted against time, a curve is formed showing four distinct phases of growth.

A- Lag phase / Acclimatization phase

B- Log phase / exponential phase / Logarithmic phase

C- Stationary phase

D- Death phase / Declined phase

Lag Phase

Immediately after transferring into a fresh medium growth does not take place but only after a period of time called the 'lag phase'. This period may be brief or extended. There is no significant increase in the number of cells. However, cell growth occurs as indicated by increase in cell mass. This stage represents a period of active growth without cell division and the cell contents prepare for the cell division by extensive macromolecular synthesis. The length of the lag phase depends on a variety of factors such as the age of the inoculum, the composition of the growth medium and the environmental factors such as temperature, pH and aeration etc. At the end of lag phase each organism divides. However, since not all organisms do not complete the lag period simultaneously, there is a gradual increase in the population until the end of this period when all cells are capable of dividing at regular intervals. If an exponentially growing culture is inoculated into the same fresh medium under the same conditions of growth, a lag is not seen and exponential growth continues at the same rate. However, if the inoculum is taken from a old (stationary phase) culture

and inoculated into the same medium a lag usually occurs even if all of the cells in the inoculum, are alive. This is because the cells are usually depleted of the various essential coenzymes or other cell constituents and time is required for synthesis. A lag occurs when the cells are damaged by treatment with heat, radiation or toxic chemicals due to the time required for the cells to repair the damage. A lag is observed when the cell population is transferred from a rich medium to a poorer one. This occurs since cells must have a complete complement of enzymes for the synthesis of the essential metabolites not present in that medium on transfer to a new medium, time is required for synthesis of new enzymes.

Log Phase

Most unicellular microorganisms grow exponentially but rates of exponential growth vary greatly. The rate of exponential growth is influenced by environmental conditions (temperature, aeration, composition of culture medium) as well as by characteristics of the organism itself. During exponential phase cells are in a steady state. New cell material is synthesized at a constant rate but the new material is itself catalytic and the mass increases in an exponential manner. This continues upto a point when one or more nutrients in the medium become exhausted or toxic metabolic products accumulate and inhibit growth. For aerobes, once cell population reaches 1 X 107 cells /ml, the growth rate will decrease unless O2 is forced into the medium by agitation or bubbling in air. When the cell concentration reaches 5 X 109 / ml the rate of oxygen diffusion cannot meet the O2 demand even in an aerated medium and growth is progressively slowed. The time taken for log phase is different for different microorganisms. Some bacteria take 20-30 minutes to grow, some soil bacteria take about 60-150minutes. Bacteria like *Nitrisomonas, Nitrobacter* normal few hours, *Mycobacterium tuberculosis* take about 12-24 hours to grow. Metabolites which are produced during log phase are known as Primary metabolites. Primary metabolites are producing only during this phase and are required for cell division and growth. It is possible to maintain a bacterial culture continuously in exponential phase for a required period of time provided that the fresh medium is supplied and toxic products or metabolic wastes accumulated in the medium are removed. Such continuous culturing is possible by devices known as ‘chemostat’ and ‘turbidostat’. The continuous culture methods have been extremely useful both for genetic and biochemical studies. This condition is obtained by growing the bacteria in continuous culture, a culture in which nutrients are supplied and end products are removed continuously. These cultures do not represent synchronous cultures since these do not contain cells that are physiologically identical. In chemostat fresh medium is added continuously at a given rate to a growing culture and excess volume thus generated is removed with an over

flow mechanism. The level of growth is controlled by maintaining a fixed, limiting concentration of a particular nutrient in the medium. The concentration of media is steadily maintained so growth rate adjusts automatically to dilution rate.

In turbidostat, a photoelectric device continuously monitors the cell density or turbidity of the culture vessel and controls the dilution rate to keep the constant turbidity either by increasing or decreasing the exchange of medium accordingly. In a photostat, which is used to get steady state cultures of photosynthetic organisms, growth rates can be controlled by controlling the light supply.

Stationary Phase

In a closed system, exponential growth cannot occur indefinitely. If a single bacterium with 20 minutes generation time continued to grow exponentially for 48 hours, it may produce a population that weighs about 4000 times the weight of the earth. In fact a single bacterial cell weighs about one trillionth of a gram. It does not happen in the nature since the growth is limited or ceased either due to the exhaustion of nutrients or due to the accumulation of toxic products. In most cases, however, cell turnover takes place in the stationary phase. There is a slow loss of cells through death, which is just balanced by the formation of new cell through the growth and division. When this occurs, the total cell count slowly increases although the viable count stays constant. Some spore forming bacteria, form endospores when they reach stationary phase if they are resistant to lysis or death. In such cases the number of viable cells will remain constant after attaining the stationary phase and a phase of decline or death may not be seen. Certain cell metabolites called secondary metabolites are produced primarily in the stationary phase. Secondary metabolites include toxins, antibiotics, sterols etc. These products inhibit the growth and survival of other microorganisms and may give competitive advantage to the producer of Secondary metabolites.

Death Phase

If incubation continues after a population reaches the stationary phase, the cells may remain alive and continue to metabolize, but often they die. If the latter occurs, the population is said to be in the death phase. The total count may remain constant while the viable count slowly decreases. In some cases death is accompanied by cell lysis leading to decrease in total count. As there is no food materials available, accumulation of toxic material leads to death of microorganisms. The cells start dying exponentially and hence we can see a sharp declined in the growth curve. After a majority of cells are dead, a small number of survivors may persist for months or even years which may be due to the growth of a few cells at the expense of nutrients released from cells that die and lyse. The terms lag, exponential,

stationary and death phases do not apply to the individual cells but only to populations of cells. Transitional periods between growth phases indicate that not all the cells are in exactly the identical physiological condition toward the end of any given phase of growth. Time is required for some cells to catch up with others. It is necessary to note that during some phases of growth the cells are young and actively metabolizing while during others they are dying, so that there may be enormous structural and physiological differences between cells harvested at different times. Physical conditions and chemical substances may also have profound effect on organisms in different phases of their growth. Generally, cells in the log phase of growth are the most uniform and in a more clearly defined condition than others and therefore used for physiological and metabolic studies.

Synchronous growth

It is very desirable to have an entire population of cells in the same stage of their growth cycle, for studying cell growth, organization and differentiation. It is not possible to analyze a single bacterial cell. Results from analysis of culture wherein all cells are in same stage of growth can be interpreted as that for a single cell. Under ordinary conditions, in an exponentially growing culture, only a small percentage of cells are actively dividing during at any one point of time. A growth pattern wherein every cell in a culture is in the same metabolic state and divides at one time is defined as synchronous growth. Therefore the increase in cell number is rather step wise than continuous. The synchrony generally lasts for only a few generations, may be 3-4 divisions, since even the daughters of single cell soon get out of phase with one another and cell division becomes random. In most bacterial cultures, especially, in log phase the bacteria are in various stages of cell division and hence, it becomes difficult to understand the properties of microorganisms. To overcome this problem, microbiologists developed cultures in which the bacteria grow synchronously.

When the bacteria grow synchronously, a synchronous population can be generated by maintaining the physical conditions or by changing the environment of the culture or by manipulating the chemical composition of the culture medium. Synchrony can be achieved by inoculating the cells and maintaining the culture at sub optimal temperatures for some time so that these cells will metabolize slowly but do not divide. But when the temperature is raised to optimum, all the cells will undergo a synchronized division. Another method to have a synchronous culture is to separate the smallest cells inlog phase culture by filtration or by differential centrifugation, which are reasonably well synchronized with each other as these cells were just divided before their separation by filtration.

Diauxic growth

In a medium containing two carbon sources, bacteria such as *E. coli* display a growth curve, which is called 'diauxic'. This phenomenon was demonstrated by J. Monod. When *E. coli* is supplied with a medium containing glucose and lactose, glucose is utilized first and only after depletion of glucose in the medium, the second C source, lactose is utilized for its growth. This happens because of the fact that the glucose metabolizing enzymes are always present in the cells (constitutive enzymes) irrespective of whether glucose is present in the medium or not, but not lactose metabolizing enzymes which are synthesized in the cell only in presence of the substrate, lactose. The *E.coli* preferentially utilizes the glucose as the enzyme is already present in the cell and therefore does not synthesize the lactose metabolizing enzymes. Since there is no alternative for *E. coli* to utilize lactose, after the exhaustion of glucose in the medium, it utilizes lactose. The time lapse between exhaustion of glucose and utilization of lactose, required for the synthesis of enzymes, for lactose utilization, is the second lag period that is observed in diauxic growth curve.

CHEMOAUTOTROPHY

Chemoautotrophs can grow in a mineral medium, during carbon from C02 & energy from the oxidation of inorganic compounds. Some of these bacteria are capable of growing both Chemoorganotrophically & chemoautotrophically i.e., they are facultative autotrophs. Example of these types are *Alcaligenes eutrophus.* Other chemoautotrophic bacteria are obligate in nature Eg. *Thiobacillus, Nitrosomonas.* Reaction which yield energy in chemoautotrophs are the oxidation of H2, NH4 +, NO-3, S & reduced sulphur compounds and Fe++. All these oxidations, except H2 oxidation, couple electron transport to the cytochrome system & NAD+ reduction occurs by energy dependent reverse electron flow.The assimilation of CO2 in these organisms occur through the reaction of the calvin cycle. When grown chemoautotrophically, cells contain high levels of the 2 enzymes of this pathway namely carboxy dismutase, phosphoribulokinase. Depending on the oxidisable in organic substrate, the chemoautotrophic bacteria can be distinguished into following groups. Nitrifying bacteria, sulfur oxidizing bacteria, H2 oxidizing bacteria, Iron oxidizing bacteria & carbon monoxide bacteria.

Nitrifying bacteria:

Nitrification is a natural process carried out by the nitrifying bacteria occurring in soil & aquatic bodies. It involves oxidation of ammonia liberated by decomposition of nitrogenous organic matter like proteins, nucleic acids, urea etc. The oxidation takes place in 2 steps – ammonia to nitrous acid & nitrous acid to nitric acid. The acids react with metal ions to produce the corresponding salts, nitrite & nitrate. Nitrate acts as main Nsource of plants.

The 2 step nitrification carried out by two different groups of bacteria.

I. First step involving oxidation of NH_3 to nitrous acid is called nitrosification. Eg. *Nitrosomonas*. The members of this genus are highly aerobic & strictly autotrophic. The energy-yielding oxidation reaction of these bacteria can be represented as $2NH_4 + + 3O_2 \rightarrow 2NO_2 - + 4H+ + 2H_2O$

II. The second step of nitrification involves oxidation of nitrous acid to nitric acid & organisms are known as nitrifying bacteria, Eg. *Nitrococcus* $2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$

The organisms of both groups are capable of generating ATP by oxidative phosphorylation in course of electron transport through the cytochrome system of the respiratory chain & final electron acceptor is O2. ATP generated in this way is utilized for CO2 fixation by Calvin Benson cycle. The part of the ATP generated by oxidative phosphorylation is spent for driving electrons from nitrite to NAD through a reverse electron transport.

SULFUR OXIDIZING BACTERIA:-

Oxidation of elemental sulfur (SO) & various reduced sulfur compounds, like sulfide (S2-), thio sulfate (S2O3 2-) etc. takes place in soil & aquatic bodies mediated by both Eubacteria & Archae bacteria. The best known among sulfur oxidizing eubacteria are members of genus *Thiobacillus*. Some species like *T. thiooxidans, T. thioparus & T. denitrificans* are obligately chemoautotrophic while *T. novellus or T. intermedins* are facultative. Some eubacteria, designated as filamentous sulfur oxidizing bacteria belonging to the genera *Beggiatoa, Thiothrix* are able to oxidise sulfide (H2S) to elemental sulfur (S0) The anoxygenic sulfur purple & green bacteria like *Chromatium, Chlorobium* etc. are able to oxidize sulfide to sulfur. *Thiobacilli* oxidise elemental sulfur or sulfur compounds to sulfuric acid. The reactions are represented as H2S + 2O2 → 2O2 → H2 SO4 Δ SO4 2- + 2H+ 2S0 + 2H2O + 3O2 → 2H2SO4 Δ 2SO4 2- + 4H + S2O3 2- + H2O + 2O2 → 2SO4 2- + 2H+

Other chemoautotrophic bacteria include

Iron - oxidizing bacteria - *Ferrobacillus ferro oxidans,* Hydrogen oxidizing bacteria – Hydrogen oxidizing bacteria are *Alcaligenes eutrophus,* All are facultative chemoautotrophs. A number of extremophilic archaebactria can grow as hydrogen bacteria eg. *Pyrodictium* (Anaerobic, obligatelychemo autotrophic).

Importance of chemoautotrophs:

Chemoautotrophs play an important role in oxidation of reduced N and S compounds like NH3 and H2S to NO3 and SO4 respectively.Nitrates sulphates are the utilized forms of nutrients for higher plants.

PHOTOTROPHY

Phototrophy refers to an autotrophic mode of metabolism in which organisms are able to utilize light energy with the help of photosynthetic pigments and convert it to chemical bond energy in the form of ATP (photophosphorylation). Phototrophic organisms are able to form organic compounds from carbondioxide, generally through Calvin - Benson cycle. The 2 processes i.e generation of ATP by photophosphorylation & CO_2 fixation together constitute photosynthesis.

Cyanobacteria (blue green algae) carry out photo synthesis with O_2 as by product.Therefore, the type of photosynthesis is known as oxygenic. There are some bacteriawhich contain bacterio-chlorophyll & carry out photosynthesis, but with out O_2 evolution(Anoxygenic)

Photosynthesis include two processes.

1. The first process include the reactions by which light energy is absorbed by the photosynthetic pigments & transformed into chemical bond energy. These reactions are photochemical in nature & are known as light reactions.
2. The second process include enzyme – catalyzed biochemical reactions involving CO2 fixation in which light has no direct role. These reaction are called dark reactions.
3. The products of light reactions are ATP & NADH2 or NADPH2. These products are used in the dark reaction for synthesis of sugar or other organic compounds from CO2.

Photosynthetic light reactions:-

The first step in photosynthesis is the absorption of photons by a series of light harvesting pigments in pigment – protein complexes. These are called antenna – complexes. The light absorbed by the antenna system is transmitted to a reaction center. The reaction center being excited ejects energy rich electrons which are accepted by the primary electron acceptor ferredoxin. Electrons from ferredoxin are then transferred to the secondary electron acceptor as a result the reaction center remains positively charged (due to loss of electrons).Appropriate position of secondary electrons acceptor leads to an electron transport in onedirection across the membrane & proton transport in an opposite direction with the consequent generation of an electric field. The overall electron flow resulting in ATP generation in photosynthesis can be of 2 main types cyclic & Non cyclic.

BACTERIAL GENETICS: GENETIC RECOMBINATION- TRANSFORMATION, CONJUGATION AND TRANSDUCTION, PLASMIDS, TRANSPOSON

Bacterial genetics

Genetics, the term, is derived from the ancient Greek word, genetikos, " genitive", and that from genesis, "origin", a discipline of biology, is the science of heredity and variation living organisms. Actually, genetics is the study of Inheritance (heredity) and Variability of the characteristics of an organism. Inheritance concerns the exact transmission of genetic information from parents to their progeny. Variability of inherited characteristics can be accounted for by a change either in the genetic makeup of a cell or in environmental conditions.

Genes correspond to the regions within DNA, a molecule composed of a chain of four different types of nucleotides—the sequence of these nucleotides is the genetic information organisms inherit. DNA naturally occurs in a double stranded form, with nucleotides on each strand complementary to each other. Each strand can act as template for creating new partner strand. This is the physical method for making copies of genes that can be inherited.

The sequence of nucleotides in a gene is translated by cells to produce a chain of amino acids, creating proteins—the order of amino acids in a protein corresponds to the order of nucleotides in the gene. This relationship between nucleotide sequence and amino acid sequence is known as the genetic code.

Definition of Gene: A chromosomal segment that codes for a single polypeptide chain or RNA molecule. Genes are arranged linearly along long chains of DNA sequence, called Chromosome.

Inheritance in organisms occurs by means of discrete traits, called gene. This property was first observed by Gregor Mendel, who studied the segregation of heritable color traits in Pea plants, in which the plants possess either purple or white color but never bears an intermediate one between the two colors. These different, discrete versions of the same gene are called Alleles. "Law of Segregation".

The set of alleles for an given organism is called Genotype, while the observable traits of the organism are called its Phenotype.

The inheritance of one character is independent another set of character, like different color of flowers of plant dependent on different allele. This phenomenon is known as 'Law of independent assortment".

Type of Genetics: i. Mendelian and Classical genetics- deals with nature of inheritance of plants (Traits)

ii. Molecular genetics deals with DNA and RNA molecules., the constituents of chromosome and its maker.

PROKARYOTE VERSUS EUKARYOTIC GENETICS:

1. Prokaryotes are haploid, and they contain a circular chromosome. In addition, prokayotes often contain small circular DNA molecules called "Plasmids",that confer useful properties such as drug resistance. Only circular DNA molecules in prokaryotes can replicate.
2. In contrast, eukaryotes are often diploid, and eukaryotes have linear chromosome, usually more than one.
3. In eukaryotes, transcription of genes in RNA occurs in the nucleus, and translation of that RNA into protein occurs in the cytoplasm. The two processes are separated from each other.
4. In prokaryotes, translation is coupled to transcription: translation of the new RNA molecule starts before transcription is finished.

Now, let us focus on the bacterial genetics exclusively.

Bacterial Genetics: There are hundreds of thousands of bacterial species in existence on Earth. They grow relatively quickly , and most of them reproduce by binary fission, the production of two identical daughter cells from one mother cell. Therefore, each replication cycle doubles the number of cells in a bacterial population. **The bacterial Chromosome** is is a long circle of deoxyribonucleic acid (DNA) that is attached to the membrane of the cell. During replication, the chromosome is copied, and the two copies are divided into two daughter cells. Transfer of genetic information from the mother cell to offspring is called **VERTICAL TRANSMISSION.**

Beneficial mutations that develop in one bacterial cell can also be passed to related bacteria of different **lineages** through the process of HORIZONTAL TRANSMISSION. There are three main forms of horizontal transmission used to spread genes between the members of the same or different species:

a. **CONJUGATION**: Bacteria to Bacteria transfer (transfer of genes between cells those are in physical contact),
b. **TRANSDUCTION**: Viral mediated transfer (transfer of genes from one cell to another by a bacteriophage) and
c. **TRANSFORMATION**: Free DNA transfer(transfer of cell free or naked DNA from one cell to another). These forms of genetic transfer can move plasmid, bacteriophage, or genomic DNA sequences. A **Plasmid** is a small circle of DNA separate from the

chromosome: A **bacteriophage** is a virus that reproduces in bacteria by injecting its DNA; the **genome** is the total DNA of the bacterial organism.

After transfer, the DNA molecules can exist in two forms, either as DNA molecules separate from the bacterial chromosome (Episome-A plasmid which can integrate reversibly with the chromosome of its bacterial host; and it behaves as a part of the chromosome, but, also able to multiply independently of the chromosome).

ROLE OF MICROBES IN SOIL FERTILITY AND CROP PRODUCTION: CARBON, NITROGEN, PHOSPHORUS AND SULPHUR CYCLES

The soil consists of five major components. They are living organisms, organic matter, air, water and minerals. The soil is generally referred as the loose material of the earth's surface, which supports the growth of plants, bacteria, fungi, algae and protozoa, which make up for the living organisms of soil. Fertile soil is inhabited by the root systems of higher plants, by many animal forms (ex: insects, worms) and by the tremendous number of microorganisms. The type and quantity of microorganisms present in a soil vary depending upon the physicalcharacteristics and agricultural practices and other parameters such as amount and type ofnutrients, available moisture, degree of aeration, temperature and pH. Soil has great variety of microorganisms of bacteria, fungi, algae, protozoa and viruses.

Important groups of microbes and their role in fertility of soil and plant growth

Microbial population in a fertile agricultural soil

Type	***Number per gram***
Bacteria	2,500,000,000 (2.5×10^9)
Actinomycetes	700,000 (7.0×10^5)
Fungi	400,000 (4.0×10^5)
Algae	50,000 (5.0×10^4)
Protozoa	30,000 (3.0×10^4)

Bacteria:

The bacterial population of the soil exceeds population of all other groups of microorganisms in both number and variety. The types of bacteria that are present in the soil are autotrophs, heterotrophs, mesophiles, thermopiles, psychrophiles, aerobes, anaerobes, cellulose digesters, protein digesters, sulfur oxidizers, nitrogen fixers and others.

Fungi:

Hundreds of different species of fungi are present in the soil. They are most abundant in the surface soil. They decompose cellulose, lignin, and pectin. The physical structure of soil is improved by the accumulation of mold mycelium within it. Yeasts are more prevalent in soils of vineyards and orchards.

Algae:

Population is smaller than bacteria and fungi. Mostly they are present on surface or subsurface of the soil. The major types present are green algae and diatoms. The growth and activity of the initial algae and bacteria paved the way for the growth of other bacteria and fungi. The cyanobacteria play a key role in the transformation of rock to soil.

Protozoa:

Most soil protozoa are flagellates or amoebas. Their dominant mode of nutrition involves ingestion of bacteria and may be a factor in maintaining some equilibrium of microorganisms in soil.

Viruses:

Bacterial, plant and animal viruses find their way into soil, through additions of plant and animal wastes. The microbial population in the rhizosphere is considerably higher than that of root free soil and physiologically more active since they make use of the root extracts.

Soil microorganisms serve as biological agents for the conversion of complex organic compounds into simple inorganic compounds or into their constituent elements. The overall process is called mineralisation. Soil microbes also fix or remove inorganic ions or mineral and this process is known as immobilization. Both immobilization and mineralisation are important for recycling of various nutrients required by plants and animals. The major nutrients essential for plant growth are C, H, O, N, P, K and S. Many of these elements undergo constant transformations in soil through the processes of immobilization, mineralisation, oxidation, reduction etc. by the soil microorganisms.

Carbon cycle

The carbon is one of the most important elements in biological systems and a component of all cell structures, which constitute about 50% of all living organisms. The carbon undergoes different oxidation – reduction states cyclically by processes known as photosynthesis, respiration, etc. The carbon cycle revolves around CO2 fixation and its regeneration.

The ultimate source of organic carbon compounds in nature is the CO2 present in the atmosphere (or dissolved in water). The CO2 in the atmosphere is fixed both autotrophically

and heterotrophically. Green plants and algae are the most important agents of CO2 fixation. Bacteria are also capable of synthesizing organic compounds utilizing inorganic CO2 in the atmosphere.

CO2 + 2H2O (CH2O) n + H2O + O2

CO2 fixation also takes place in some heterotrophic microorganisms

CH3 – CO – COOH + CO2 HOOC – CH2 –CO –COOH

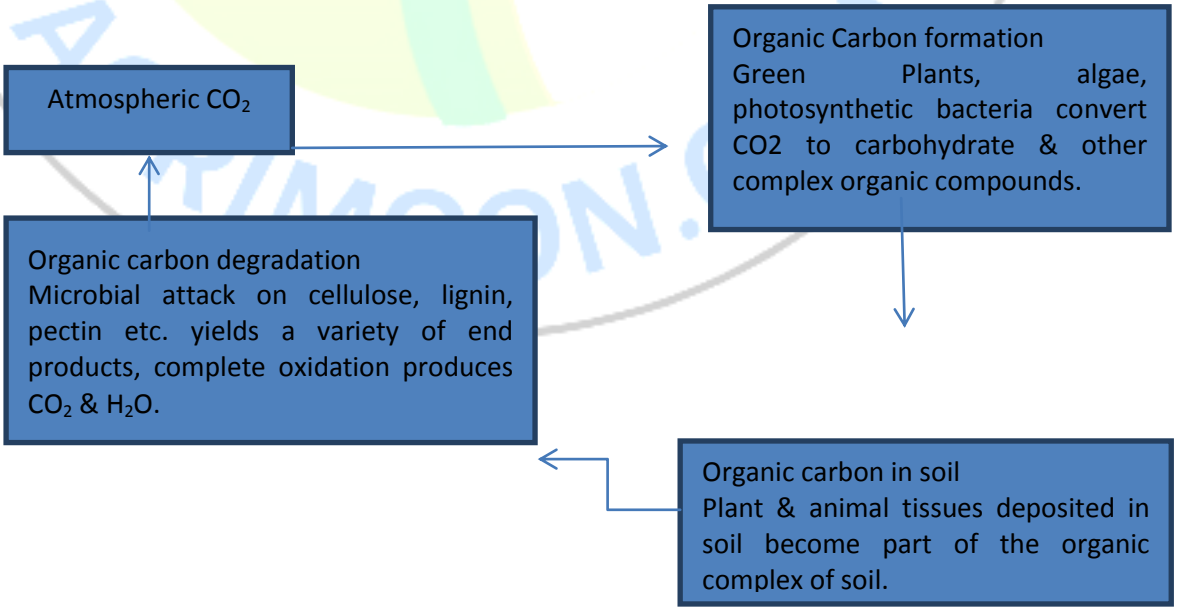
Pyruvate Oxaloacetate

Under anaerobic conditions, phototrophic bacteria fix CO2 by utilizing H2S or organiccompounds as a source of electrons.

CO2 + 2H2S (CH2O)n + 2 S + H2O

Microbes involved in redox cycle for carbon

The organic carbon compounds that are eventually deposited in the soil are degraded by microbial activity, CO2 is released in to the air and soil. Top six inches of fertile soil contains approximately 2 tonnes of fungi and bacteria per acre whose metabolic activity equals to nearly tens of thousands of human beings. The large surfaceto volume ratio of microorganisms enhance rapid exchange of substrates and waste products from the environment. Any one particular genus has a limited capacity to degrade numerous compounds present in nature but a variety of different genera of microbes together would nearly degrade almost every compound occurring in nature. Nearly 90% of CO2 in the atmosphere is accounted for by the release of CO2 on decomposition and respiration of organic compounds by bacteria and fungi.



Carbon Cycle

Nitrogen cycle

The element nitrogen is a key element of protoplasm of living cells. It exists in a number of oxidation states. Several of **t**he redox reactions of N are carried out solely by microorganisms and the microbial involvement in the nitrogen cycle is of gr**e**at importance. Thermodynamically N2 gas is the most stable form of nitrogen . Molecular N2 constitutes about 78 % of earth's atmosphere. This form is chemically inert and cannot be utilised by most living organisms. Plants, animals and microorganisms depend on a source of combined nitrogen such as NH3, $NO3^-$ or organic N compounds for their growth. A part of atm.N2 is converted into a reduced form and further into an organic form by certain free living bacteria and by some plant-microbe associations. The nitrogen cycle mainly involves transformations such as

I) Nitrogen mineralization in which nitrogen complexes are decomposed into simpler or organic forms and converted into inorganic compounds for use by plants

II) Nitrogen immobilization in which nitrogen compounds are assimilated into cellular materials.

Nitrogen Mineralization:

In the process of mineralization, proteins, nucleic acids and their components are degraded by microorganisms with the eventual liberation of ammonia and this is called ammonification. A part of the liberated ammonia is assimilated by the microorganisms themselves. The first step in the process of ammonification is the hydrolysis of proteins, nucleic acids and other organic nitrogenous compounds into amino acids (proteolysis). The amino compounds are then de-aminated to yield ammonia. Ammonification usually occurs under aerobic conditions. Protein decomposition leads to conversion of ammonia into amines and related compounds. These amines are subsequently oxidized in the presence of oxygen to release ammonia. In nature, the breakdown of nitrogenous substances is brought about by the activity of a multitude of microbial species. Almost all bacteria, actinomycetes and fungi can bring about proteolysis and the amino acids produced are utilized for the growth of these organisms.

NITROGEN CYCLE

N≡N

Denitrification/NO^-_3 respiration **Biological N fixation from**

Reduction of NO^-_3 occurs & gaseous N_2 **atmosphere(gaseous N_2**

Formed & evolved. Ex. Pseudomonas, Thiobacillus transformed to NH3-key

Intermediate

(Ex.Azotobacter, Rhizobium, BGA)

NO^-_3 formation: NO^-_3 serves as plant food Organic N-formation

NO-2 → [O] → NO^-_3 Nitrobacter Fixed N utilized by plants,

converted to plant proteins

consumed by animals

proteins formed subsequently.

NO-2 formation Many heterotrophs reduce NO-3 to NH3

NH^+_4….[O]…..NO^-_2 Soil Organic-N formed

Ex. Nitrosomonas, Nitrolobus, from the excreta of plants,

Nitrosococcus, Nitrososporaanimals & microbes-

deposited in soil.

Ammonification ← Organic N-degradatio

Nitrogen immobilization:

When plant residues or pure carbohydrates are added to the soil, there is a rapid decrease in the amount of available inorganic nitrogen which is referred as “nitrogen immobilization”. It results from the microbial assimilation of inorganic nitrogen. The process of immobilization involves the incorporation of ammonia and nitrate into microbial protein and nucleic acids and is therefore the reverse of mineralization. Mineralization and immobilization therefore run counter to each other. On the death ofmicroorganisms, the immobilized nitrogen is however, released through mineralization.

Nitrification:

In the second phase, ammonia is converted into nitrate and this process is called nitrification. Nitrification occurs in two steps; first, ammonia is oxidized to nitrite:

2 NH3 + 1 ½ O2 $NO2^{—}$ + H2O.

2 N H3 + 3 O2 2 H NO2 + 2 H2 O.

This change is brought about by chemoautotrophic bacteria of the genera *Nitrosomonas, Nitrosolobus, Nitrosococcus* and *Nitrosospira*. These bacteria obtain their energy requirement by the oxidation of NH4 + to $NO2^{-}$. Of these nitrifying organisms, *Nitrosomonas* are the most important in the soils.

Besides the chemoautotrophic bacteria, some heterotrophic bacteria such as *Streptomyces* and *Nocardia* have also been known to oxidize ammonia to nitrite. *Nitrosomonas*, first converts ammonia to hydroxylamine which is then transformed into some undefined intermediate, possibly a compound such as nitroxyl (HNO). This intermediate is then oxidized to nitrite possibly by way of Nitrous oxide as shown below:

In the second step, nitrite is oxidized to nitrate:

Nitrobacter oxidizes nitrite to nitrate and yields two electrons for each molecule of NO2 transformed..

HNO2 + ½ O2 HNO3

Certain fungi belonging to the genera *Aspergillus, Penicillium* and *Cephalosporium* can also carry out nitrification.

Denitrification

Certain bacteria are capable of using nitrate as the terminal electron acceptor under anaerobic conditions. Nitrate is reduced to nitrogen gas or nitrous oxide. This process iscalled as denitrification or nitrate respiration and leads to the loss of nitrogen from the soil. Denitrificationdepletes the soil of an essential nutrient for plant growth and therefore is not a desirablereaction. Denitrification occurs mostly in waterlogged anaerobic soils with a high organicmatter content and the ability to carry out denitrification is restricted to only certain bacteria.. Among the bacteria important in denitrification are *Thiobacillus denitrificans, Micrococcus denitrificans* some species of *Pseudomonas, Bacillus, Paracoccus, Achromobacter* and *Serratia.* The enzymes involved in various steps of denitrification reactions are called as nitrate, nitrite, nitric oxide and nitrous oxide reductases. The overall reaction is :

2 HNO3 =2 HNO2 +2NO+ N2+ N2O

Nitrate is first reduced to nitrite which is then transformed to NO. The NO is converted to N2 with N2O as intermediate. Although denitrification is an undesirable reaction from the point of view of plant nutrition, the supply of nitrogen on the earth would have got depleted and $NO3^{-}$ would have accumulated. Also, since high concentration, of $NO3^{-}$ are toxic,

denitrification is a mechanism by which some of the nitrogen is released back to the atmosphere.

Phosphorus cycle

Phosphorus is only second to nitrogen as a mineral nutrient required for plants, animals and microorganisms. It is a constituent of nucleic acids and essential for the accumulation and release of energy. Microorganisms are known to bring about a number of transformations of this element. These includes

1. Altering its solubility
2. Mineralisation of organic phosphate compounds into inorganic phosphates
3. Oxidation and reduction of phosphorus compounds.

Minaralisation and immobilization are the most important.

Mineralisation and immobilization:

The plants utilize phosphate ions and synthesize organic phosphates within the cell. The organic phosphorus of plants, animals and microorganisms is released by enzymatic hydrolysis by phosphatases released by soil microflora. These enzymes show a broad range of specificity and are grouped into two groups based on their pH optima, the alkaline phosphatases and the acid phosphatases.

Solubilization

Phosphate becomes limiting for plants growth because much of the phosphorus is in the bound form in the soil as insoluble Ca+, Fe2 + or Al^{+3} phosphates. Microorganisms produce various organic and inorganic acids and there by solubilize insoluble phosphates. Some of the fungi and a number of bacteria found in soil produce these acids and make the insoluble phosphorus available to the plants in the form of phosphates. Ex:-The species of *Bacillus, Pseudomonas, Micrococcus, Flavobacterium, Phosphobacterium, Aspergillus, Penicilluim, Fusasrium* and others. Several fungi that associate with plant roots are *Mycorrhizae,* and they help in phosphorus uptake. Some of the bacterial preparations are used as phosphatic biofertilizers for solubilisation of phosphorus in soils rich with insoluble phosphates.

Sulphur cycle

Sulfur transformations are even more complex than those of nitrogen due to the variety of oxidation states of sulfur. Some sulfur transformations occur at significant rates chemically as well as biologically. To be useful, sulfur has to be first oxidized or reduced in the soil. It

occurs both in organic (sulfur amino acids and vitamins) as well as in inorganic form (S, H_2S, SO_4^{2-}, etc) and is readily metabolized. These transformations are

1. Decomposition of larger organic sulfur compounds to smaller unites and their conversion into inorganic compounds
2. Microbial associated immobilization
3. Oxidation of inorganic ions and compounds such as S^{2-}, $S_2O^{3\ 2-}$, S
4. The reduction of SO_4^{2-} and other ions to sulphides.

Plants utilize sulfur in the form of sulphates and reduce to H2S within cells to be utilized in synthesis of amino acids, vitamins etc. Animals obtain their sulfur by feeding on plants. When plant, animal and microbial proteins are degraded, the sulfur is released from amino acids and accumulates in the soil. This is further oxidized to SO_4^{2-} under aerobic conditions. Under anaerobic conditions the sulfur accumulated in the soil is converted to H_2S. The biological oxidation of elemental sulfur and inorganic sulfur compounds such as H_2S, SO^{3-} and $S_2O_3^{2-}$ is brought about by chemoautotrophic and photosynthetic bacteria. The oxidation of H2S is characteristic of pigmented photosynthetic bacteria, which use H_2S as an electron donor in photosynthesis. Members of *Thiobacillus* genus oxidize elemental **S**. Heterotrophic bacteria actinomycetes and fungi are also reported to oxidize sulfur compounds. Bacillus, *Pseudomonas, Arthrobacter* and *Flavobacterium* oxidize S to SO_4^{2-} REACTION: S SO^{3-} SO_4^{2-}

Under anaerobic conditions,

$SO_4^{2-} \longrightarrow SO_3^{2-} \longrightarrow S2O_3^{2-} \longrightarrow S^{2-}$

Bacillus, Pseudomonas, Desulfovibrio reduce SO_4^{2-} to H_2S.

Dissmilation of S as H_2S and its release into the atmosphere far exceeds the total amount of H_2S produced from all other pollution sources.

BIOLOGICAL NITROGEN FIXATION:

A variety of procaryotic organisms are known to have the ability to reduce atmospheric nitrogen and fixation of the inert atmospheric elemental nitrogen by microorganisms through a reductive process called "Biological Nitrogen Fixation". The conversion of molecular nitrogen into ammonia by microorganisms is known as Biological nitrogen fixation. In 1838, Boussingault showed that leguminous plants can fix atmospheric nitrogen and increase the nitrogen content of the soil. This observation led to better understanding of the practice of crop rotation involving legume crops. Beijerinck later identified the bacteria that are associated with roots of leguminous plants as Rhizobia. By the end of 19 th century, many free living aerobic and anaerobic bacteria were found to have the ability to fix atmospheric N2 the associative symbionts are a new class recognized recently to have the ability to fix atmospheric N2 in association with the roots of grasses and cereal plants. Several free-living blue green algae were found to fix atmospheric N2. It was estimated that global – N input was nearly 2.6 X 1011 Kg N per year with biological nitrogen fixation processes contributing for nearly 70%.

Nitrogen fixation on earth's surface through various processes

In symbiotic systems biological nitrogen fixation leads primarily to ammonia formation which is assimilated into plant proteins. In non- symbiotic systems first N2 is converted to microbial proteins and after the death of microbes, proteins decompose, nitrification takes place and then $NO3^{-}$ will be taken up the plants. The enzyme, nitrogenase, which reduces atmospheric N2 to $2NH_4^{+}$, has been found to be present in all most all of the nitrogen-fixing bacteria. This enzyme has been fairly well characterized and the enzymes from different systems have common properties allowing a unified description of a single nitrogenase. Nitrogenase contains two components. Component - I is designated as Mo-Fe protein (nitrogenase) and component-II is designated as Fe–protein (nitrogenase reductase). Both the components are oxygen sensitive. Mo-Fe protein consists of 4 sub–units whereas Fe protein consists of 2 sub–units.

The essential reactants in the bacterial nitrogen fixation process are

1. Components I & II
2. A strong reducing agent,
3. ATP molecules,
4. A regulating system for NH3 production and utilization,
5. A system that protects the N2 fixing system from inhibition by O2.

The overall biochemical reaction for nitrogen fixation can be expressed as:

Nitrogenase

$N2 + 6e^{-} + 12\ ATP + 12\ H2O\ 2NH_4^{+} + 12ADP + 12Pi + 4H +.$

The amount of nitrogen fixed by various systems can be measured by N15 method and acetylene reduction method. The latter method is simple. rapid, relatively inexpensive technique now widely used to measure nitrogen fixation. The test is based on the observation that the nitrogen-fixing enzyme (nitrogenase) interacts with triple bonded compounds. eg. Acetylene to form ethylene as follows.

$HC \equiv CH\ 2e^{-}\ H2C \equiv CH2$

Nitrogenase

Acetylene ethylene

The comparable reaction with nitrogen is

$N \equiv N\ 6e^{-}\ 2NH3$

Nitrogenase

The technique involves exposing the specimen to acetylene in a suitable vessel and after a period of incubation, the amount of ethylene produced is measured by gas liquid chromatography. The amount of ethylene produced is a measure of nitrogenase activity. In recent years efforts are being made to improve biological nitrogen fixation by modification of the plant system or by introduction of N_2 fixing genes into plants or by improving the efficiency of N_2 fixing bacteria.

Some prominent biological nitrogen fixing systems and examples

A. Symbiotic:

Root nodules and /or stem nodules, Legumes + *Rhizobia,* leaf nodules etc. Non legumes + Actinomycetes (Dicotyledons) + (Frankia*)*

Others: Azolla Fern + Blue green algae

Lichens Fungus + Blue green algae

B. Non symbiotic:

Cynobacteria (BGA): *Anabena spp., Nostoc spp,Gleotrichia spp, Oscillatoria spp.*

Phototrophic bacteria: *Rhodopseudomonas, Chromatium, Chlorobium*

Chemotrophic bacteria*: Azotobacter chroococcum, Azospirillum lipoferum* (Associative symbiotic bacterium)

BLUE GREEN ALGAE, AZOLLA AND MYCORRHIZA (AM FUNGI)

Cyanobacterial:

The Blue green algae are also being produced on large scale and used as biofertilizers in rice cultivation. The efficient strains are cultured in open plots with water containing adequate amounts of mineral nutrients such as phosphate and molybdate. After sufficient growth is obtained, the algal mat is collected from the plot, dried and used as inoculant. Cyanobacteria can also be grown directly in the paddy field before the transplantation of rice plants. Azolla, a symbiotic association of a small fern with blue green algae is also used as a biofertilizer and green manure for rice cultivation.

Mycorrhiza

Some fungi form a symbiotic association with roots of higher plants faciliatating uptake of plant nutrients, particularly of those which are less mobile. This association is known as "Mycorrhizal association". There are two types of Mycorrhizal association:

(i) Ectotrophic mycorrhizae- where the fungus form a mantle or sheath around the root surface (called Harting net) and where the mycelium develops intercellularly. The fungi which form this type of association are species of *Boletus, Amenuta*, etc.

(ii) Endomycorrhizae- where the fungus develops intracellularly in the root without forming Harting net. In this association, the penetration of roots cells is charecterized by the formation of terminal spherical structure called vescicles which contain oil droplets and phosphorous. This type of mycorrhiza is called 'vesicular Arbuscular mycorrhiozae' (VAM) and is of agricultural significance particularly in P- deficient soils where the phosphorous in the vesicles diffuse out into the cytoplasm and is taken up by the plant. Fungi belon to this groups are *Glomus, Gigaspora*, etc.

The beneficial effect of these fungi on nutrient uptake has been attributed to three factors.

(i) Increased absorption of available nutrients from soil as the fungus changes root morphology which results in the larger root surface available for nutrients absorption. Fungal filaments also act as absorption surface.

(ii) Increasing the nutrient availability by solubilizing insoluble nutrients like phosphorous which thus become available to plants.

(iii) Increasing the nutrients mobility due to faster intracellular nutrient mobility and mobilizing nutrients from the soil mass not visited by the root system but traversed by the Mycorrhizal hyphae.

RHIZOSPHERE AND PHYLLOSPHERE

Rhizosphere:

Rhizosphere can be defined as the region extending a few millimeters from the root surface in which the microbial population of soil is influenced by the chemical activities of plant roots. The rhizosphere differs from the bulk soil because of the activities of plant roots & their effect on soil organisms. A major characteristic of the rhizosphere is the release of organic compounds into the soil by plant roots. These compounds called root exudates makes the environment different in rhizosphere and bulk soil. The exudates increase the availability of nutrients in the rhizosphere & also provide a carbon source for heterotrophic microorganisms. The population of organisms in the rhizosphere can be 500 times higher than in bulk soil. Organisms in the rhizosphere can affect the plant roots by altering the movement of carbon compounds from roots to shoots. Many microorganisms are beneficial and are called Plant growth promiting rhizobacteria(PGPR). Various root microbes association can increase nutrient uptake by plants in nutrient poor environment such as symbiosis (eg. Mycorrhizal or Rhizobia) & specific association (Associative N2 fixing bacteria with grasses etc – *Azospirillum)* . Some microorganisms produce hormones that stimulate plant growth and some microorganisms are antagonistic to plant pathogens. But some soil microorganisms are pathogenic & attack living plant roots.

Rhizoplane:-

The rhizoplane is the surface of the plant roots in the soil. The rhizoplane is the site of the water & nutrient uptake & the release of exudates in to the soil. As roots grow they cast dead cells & navigate around the soil particles making the rhizoplane highly irregular, blurring the dividing line between the root surface & soil.

Phyllosphere:

The region on the leaf surface where microorganisms are present abundantly. The leaf surface microbes may perform an effective function in controlling the spread of air borne microbes inciting plant disease. Resistance to disease causing microbes has also been attributed to fungistatic compounds secreted by leaves such as malic acid etc. Phyllosphere bacteria are often pigmented due to direct solor radiation.Any change in phyllosphere, affects plant growth which in turn affects the physiological activity of root system. Such changes in the root results in an altered pH & spectrum of chemical exudation causing a change in rhizosphere microflora. Thus there is a link between phyllosphere microflora and rhizosphere microflora. There is a continuous diffusion of plant metabolites from leaves which support the microbial growth & in turn these microbes protect the plant from pathogens.

MICROBES IN HUMAN WELFARE: SILAGE PRODUCTION, BIOPESTICIDES, BIOFUEL, BIOFERTILIZERS PRODUCTION AND BIODEGRADATION OF AGRO-WASTE

Role of microbes in fermentation

Cagnaird Latour; Theodor Schwann; F. Kutzing independently showed that microbes are involved in fermentation of sugar to alcohol. **Louis Pasteur** continued his work and found that fermentation of fruits and grains, resulting in alcohol was brought out by microbes. Pasteur suggested that good quality fermented products can be obtained by selecting proper microbe. The otherunfavorable microbes can be avoided by heating the fruit juice at 62.8^0C for 30 min. This process is called ***Pasteurization*** and is widely used in fermentation industries. Thi short heating process kills pathogenic and spoilage microorganisms but does not sterilize the liquids completely by keeping the quality intact. During his studies on the butyric fermentation, Pasteur discovered the existence of forms of life, which can live only in the absence of free oxygen. He introduced the terms aerobic and anaerobic to designate life in the presence and absence of oxygen respectively. Pasteur described that fermentation is life without air. Some strictly anaerobic microorganisms such as the butyric acid bacteria are dependent on fermentative mechanisms to obtain energy. Most of the organisms require oxygen to oxidize organic compounds to CO2. Such oxygen linked biological oxidation known as aerobic respiration provides energy that is required for maintenance and growth.

Facultative anaerobes: Many other microorganisms including certain yeasts are facultative anaerobes which have two alternative pathways of energy yielding mechanisms – in the presence of oxygen they employ aerobic respiration – in the absence of oxygen, they employ fermentation. Yeast Ex: Sugar Alcohol + CO2 , No O2; yeast Sugar CO2, no Alcohol O2

The above process was demonstrated by Pasteur. Fermentation is a less efficient energy yielding process than aerobic respiration, because the part of the energy present in the substance degraded is still present in the organic end products. At the same time **Ferdinand Cohn** demonstrated that certain bacteria could produce spores, which are heat resistant.

Lecture

Fermentation

In the middle of the 19th century, Fermentation is said to be purely a microbiological process by Louis Pasteur. He studied various types of fermentations and demonstrated that each

particular type of fermentation occur by the act of specific type of microorganism. The substances produced during fermentation are very useful natural products. Distillers, cheese makers, bakery exploit this property for the preparation of fermentation products on industrial scale. Fermentation can be defined as a reaction in which organic compounds serve both as electron donors & electron acceptors. It is also defined as an internally balanced ATP generating oxidation – reduction process in which an organic compound i.e., a substrate is partially oxidized or partially reduced. Since there is no net oxidation in a fermentation, the number of moles of C, H & O remain same in the products as in the substrate as it occurs in the conversion of glucose to lactic acid or ethanol. Energetically fermentations are poor energy yielding processes. Fermentation differs from anaerobic respiration in that, in the later oxygen is replaced by an inorganic electron acceptor such as NO-3. The term fermentation is loosely used to denote any process which microorganisms are involved even if the process involved strong aeration and is respiratory in nature as in antibiotic fermentations. Hence, fermentation is broadly defined as any large scale conversion carried out either by microorganisms or by their products like enzymes. Microorganisms ferment various sugar compounds and the type of fermentations are basically ethanol, lactic acid, mixed acid, propionic acid, butyric acid, mixed amino acid and butanediol types. C6 H12 O6 2 C3 H6 O3 Or C6 H12 O6 2 CH3 CH2 OH + 2CO2 Glucose ethanol Carbon dioxide. In fermentation, the end product of glucose break down via EMP or EDP is pyruvic acid which is transformed into various compounds, depending on the organisms. Pyruvic acid acts as H acceptor & regenerates NAD while getting itself reduced to Lactic acid. Successful production of organic chemicals using microorganisms is dependent on several factors such as the organism, the raw materials and the cultural conditions. A desired end product is obtained in large quantities by utilizing a suitable microbe and a cheap raw material. The improvement of these processes is possible either by manipulating the cultural conditions of fermentation or addition of a few chemicals in the medium or by genetic manipulation of organisms including mutations, hybridization etc.

Components of a fermentation includes Substrate / raw materials; Microorganisms; Bioreactor/ fermentor; End product-Sugar *Saccharomyces cerevisiae* Alcohol

Fermentation conditions includes pH, temp, time, aeration etc.

List of different types of fermentation:

1. ETHANOL FERMENTATION:-

In alcoholic fermentation, pyruvate which is first activated, is then converted by pyruvate decarboxylase into acetaldehyde & CO2. Acetaldehyde is then reduced to ethanol in a NAD linked reaction involving alcohol dehydrogenase. This fermentation is a major pathway in some yeasts *Saccharomyces cerevisiae* but is not important in bacteria.

2. **LACTIC ACID FERMENTATION:-**

In this fermentation, a NAD linked lactic dehydrogenase reduces pyruvate to lactate. Lactic acid bacteria such as *Lactobacillus cascei*, *Streptococcus cremoris* etc. carry out lactic acid fermentation in which lactic acid is the only end product. Lactic acid bacteria consists of 2 sub- groups based on the nature of the product they form. They are Homolactic fermentors and heterolactic fermentors. In both, alcohol and lactic acid type of fermentation the net yield of ATP is 2 per mole of Hexose. Two moles of ATP are consumed in the formation of a hexose diphosphate from glucose & 4 moles of ATP are subsequently produced, the net yield is 2 moles of ATP per mole of hexose.

3. PROPIONIC ACID FERMENTATION:

In this fermentation, pyruvate is first carboxylated to yield oxaloacetate which is then reduced to succinate & then decarboxylated to propionate. Pyruvic acid→ Oxaloacetic acid → Succinic acid → Propionic acid. Propionibacterium is exclusively used in the preparation of Swiss cheese. This type of fermentation mostly occur in the rumen (stomach) of ruminants and grain eatinganimals.

4. MIXED ACID FERMENTATION

This fermentation is characteristic of most members of enterobacteriaceae which dispose a part of glucose through lactic acid fermentation & a part through another fermentation in which pyruvate is split without net oxidation or reduction to an acetyl group & formic acid. The major end products are pyruvate, succinate and formic acids. *E.coli*

5. ACETONE – BUTANOL TYPE:-

This kind of fermentation is carried out by strict anaerobes such as *Clostridium*. The glucose is initially cleaved to H2, CO2 and two carbon fragments. Two such fragments are condensed to yield acetoacetyl CoA that is de-carboxylated and or reduced to acetone, isopropanol, butyrate and n-butanol in varying proportions.

6. 2,3 BUTANE DIOL TYPE: -

This is carried out by *Enterobacter* sp. By the condensation of two lactate molecules, acetolactic acid is produced which is further converted to butane diol. The glycolytic pathway of glucose is the most common pathway in microorganisms and reflec a common evolutionary path and selection of most effective mechanisms in differentmicroorganisms.

7. MIXED AMINO ACID TYPE: -

This type of fermentation occur during putrefactive processes in which certain amino acids serve as electron donors and while others serve as acceptors. A large number of *Clostridia* putrefy protein rich substrates and produce unpleasant odours.

Microbial Composting, biodegradation, Biogas production

Fermentation methods are of great service in the treatment of industrial wastes or domestic sewage when the amount and character of the organic matter is such as to serve as a substrate for bacterial or other microbial action. Such fermentations are sometimes based on an effort to utilize as fully as possible the fermentable material with production of some gaseous or other product, sush as methane, which might be utilized as a source of heat energy in the industrial operation of plant. Microbial conversion of waste materials such as agricultural, industrial and domestic wastes, by microorganisms into inorganic compounds is called biodegradation. For successful survival of mankind on earth, recycling of organic and inorganic materials is essential. Microorganisms degrade various organic wastes by various biochemical processes and purify them to a stage of reutilization. The biogas production has gained importance in view of developing alternate sources of energy. The environmental conditions in our country are very suitable for the production of biogas.

Composition of Biogas:

Biogas is a mixture of methane (50-60%), carbon dioxide (30-40 %), hydrogen (5-10 %), H2S and nitrogen (traces), produced from the anaerobic digestion of animal, plant wastes or any cellulose containing waste material.

Microbiology of biogas production:

The digester used for biogas production is called a biogas plant. A typical biogas plant using cow dung as raw material consists of a) digester and b) gas holder. The digester is of continuous type, which is fed with a definite quantity of wastes at regular intervals so that gas production is continuous and regular. The nature of fermentation in digester is anaerobic.

There are three phases in the anaerobic fermentation of organic matter to methane.

1. The hydrolytic bacteria, which catabolize carbohydrates, proteins, lipids other components of biomass to fatty acids, H_2 and CO_2.

2. The acidogenic bacteria which catabolize certain fatty acids and natural end products of group one to volatile fatty acids like propionate, acetate, formate, carbon dioxide, and hydrogen.

3. The methanogenic bacteria which utilize acetate, CO2 and H2 to produce methane

The hydrolytic and acidogenic groups of bacteria include facultative as well as strict anaerobes like *Cellulomonas, Clostridium, Bacillus, Bacteroids, Ruminococcus, Eubacterium* etc. While the methanogenic bacteria includes *Methanosarcina, Methanothrix, Methanobacterium* and *Methanospirillum.*

The methanogenic phase is strictly anaerobic and during this phase organic carbon is converted into microbial mass, CO2 and methane. These bacteria are sensitive to pH and the optimal pH for the methane production is 6.8 to 7.2. If pH drops to 6.6 or below there is an inhibition of methanogenesis.

Biogas manures:

The material after fermentation and production of biogas are called as biogas manures and they can be used as manures to different crops. From the plant's perspective, biological control can be considered a net positive result arising from a variety of specific and non-specific interactions. The types of interactions are referred as parasitism, antagonism, competition, and predation etc.

• Requirement of favourable environmental conditions for the pathogens to act, multiply and execute its mode of action.

• Potential biological control agents need to be subjected to extensive testing and quarantine before release into any new environment.

Microbial insecticides

Many microorganisms such as mycoplasma, bacteria, fungi and protozoa are pathogenic to insects. **Microbial preparations and formulations in the insect pest control programme are known as microbial insecticides.**

List of different microbial insecticides and their host range are given below:

Microbial insecticides	Host Range
1. Bacterial insecticide	
a. Bacillus thuringiensis	Silkworm, Cabbageworm, Sweet potato, Leaf worm, Tobacco cutworm, Rice leaf roller etc.
b. Bacillus papillae	Japanese beetle, which causes damage to

	number of trees and shrubs.
c. *Bacillus sphearicus*	Mosquitoes.
2. Fungal insecticides	
a. *Beauveria bassiana.*	Potato beetle.
b. *Metarrhizium anisopiiae*	Rhinocerus beetle, Black rice bug.
3.	

ADVANTAGES:

1. They donot cause pollution hence they are ecofriendly
2. They are more specific and hence donot affect beneficial insects

DISADVANTAGES:

1. Immediate effect is not seen owing to the incubation period of the microorganisms in the body of insect.
2. The narrow host range of microbial insecticides is disadvantageous in practical insect pest control.
3. The contact infection occurs only in the case of fungal infections and in the case of others, insects should necessarily ingest leaves, coated with microbial insecticide before being infected

Microbial insecticide preparations of bacteria, protozoa, and virus are available in the form of dusts, wettable powders and water dispersible emulsions.

Applications

Control of plant diseases.

1) *Bacillus cereus* strains –produce the antibiotic zwittermicin –protect tomato and alf-alfa plants from various soil born fungi –*Phytophthora* and *Pythium*

2) *P. fluorescens*, prevents bacterial blotch by competing with *P. tolaasii*

3) *Trichoderma viridae*: against Root Rot, Stem Rot, Wilt, Lead Spot, Early & Late Blights, Tikka Disease, Downy Mildews, etc. of different crop plants.

Microbial agent for control of plant diseases

From the plant's perspective, biological control can be considered a net positive result arising from a variety of specific and non-specific interactions. The types of interactions are referred as parasitism, antagonism, competition, and predation etc.

Parasitism is a symbiosis in which two phylogenetically unrelated microorganisms coexist over a prolonged period of time. In this type of association, one organism, usually the

physically smaller of the two (called the parasite) benefits and the other (called the host) is harmed to some measurable extent. The activities of various hyperparasites, i.e., those agents that parasitize plant pathogens, can result in biocontrol. And, interestingly, host infection and parasitism by relatively avirulent pathogens may lead to biocontrol of more virulent pathogens through the stimulation of host defence system.

Antagonism (ammensalism) one microorganism produces a substance that is inhibitory to other microbial population results in a negative outcome for later one. Production of oxygen may alter the population of obligate aerobes. Ammonia produced during decompositions of proteins and amino acids at concentrations inhibitory to nitrite oxidizing populations of *Nitrobacter*.

Competition within and between species results in decreased growth, activity and/or fecundity of the interacting microorganisms. For example biocontrol can occur when non-pathogens compete with pathogens for nutrients in and around the host plant.

Predation refers to the killing of one microorganism by another for consumption and sustenance. While the term predator typically refer to animals that feed at higher trophic levels in the macroscopic world, it has also been applied to the actions of microbes, e.g. protists, and mesofauna, e.g. fungal feeding nematodes and microarthropods, that consume pathogen biomass for sustenance. E.g. *Dinidium nasutum* preys on *Paramecium* Biological control can result in varying degrees from all of these types of interactions, depending on the environmental context within which they occur. Significant biological control, as defined above, most generally arises from manipulating mutualism between microbes and their plant hosts or from manipulating antagonism between microbes and pathogens.

ADVANTAGES

• Environment friendly and leave behind no toxic residues.

• Target specific pathogen and avoids unnecessary effect on beneficial microflora and microfauna.

• Most of them are easily culturable in the lab, with minimum space.

• Inexpensive to produce large quantities of inoculum.

• Its mimicry of nature by releasing them into an open environment.

• Biological control could reduce the use of many pesticides and herbicides hence, which could eliminate the overuse of chemicals by farmers and further reduces cost of cultivation.

DISADVANTAGES

• Necessity for careful and correct time of application.

• Host specificity of most pathogens, narrows down its use.

• Necessity to maintain a pathogen in a viable condition.

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• Difficulty in producing some obligate and facultative pathogens on a large scale.

• Requirement of favourable environmental conditions for the pathogens to act, multiply and execute its mode of action.

• Potential biological control agents need to be subjected to extensive testing and quarantine before release into any new environment.

APPLICATIONS

Control of plant diseases.

1) *Bacillus cereus* strains –produce the antibiotic zwittermicin –protect tomato and alf-alfa plants from various soil born fungi –*Phytophthora* and *Pythium*

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Biodegradation of Plastics

Lack of degradability and the closing of landfill sites as well as growing water and land pollution problems have led to concern about plastics. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic wastes has assumed increasing importance in the last few years. Awareness of the waste problem and its impact on the environment has awakened new interest in the area of degradable polymers. The interest in environmental issues is growing and there are increasing demands to develop material which do not burden the environment significantly. Biodegradation is necessary for water-soluble or water-immiscible polymers because they eventually enter streams which can neither be recycled nor incinerated. It is important to consider the microbial degradation of natural and synthetic polymers in order to understand what is necessary for biodegradation and the mechanisms involved. This requires understanding of the interactions between materials and microorganisms and the biochemical changes involved. Widespread studies on the biodegradation of plastics have been carried out in order to overcome the environmental problems associated with synthetic plastic waste.

The marketing claims which can be made about Oxo-biodegradable plastics may vary from country to country and in some regions may be legislated through trade and consumer bodies.

In some cases these 'green claims codes' may restrict how the benefits of oxo-biodegradable technology can be described or depicted in marketing claims. It is therefore the responsibility of the end user to satisfy themselves that they are marketing the product in line with the market destination for which the product is intended. Wells Plastics Limited cannot be held responsible for the incorrect marketing of end products by users of the technology and we recommend that all marketing statements are checked by local lawyers familiar with the local legislation. Synthetic plastics are emerging environmental contaminants that have been found to accumulate within marine waters worldwide. in marine environments, microorganisms function as pioneering surface colonizers and drive critical ecosystem processes including primary production, biogeochemical cycling and the biodegradation of anthropogenic pollutants. this paper reviews the current knowledge on the biodegradation of synthetic plastics by microorganisms. the microbial biodegradation of plastic materials is a complex phenomenon that includes several steps that are described here.

Introduction

Colonization of plastic marine debris by microorganisms has been firstly reported in the 1970s, where authors mention diatoms and other microbes on the debris (Carpenter *et al.* 1972, Colton *et al.* 1974). In marine waters, plastic debris represent a novel ecological habitat for microorganisms since it entered the consumer arena less than 60 years ago, acting as new floating type of particles for microbial colonization and transportation. Plastic has become the most common form of marine debris and it presents a major and growing global pollution problem. In the North Western Mediterranean Sea, plastics were found at concentrations of up to 3.6 10^5 pieces/km^2 (Collignon *et al.* 2012), which is equivalent to what was found in the "great Pacific garbage patch" (5.0 10^5pieces/km^2 were found in the North Atlantic Subtropical Gyre, Law *et al.* 2010). Particles may serve as a niche for microorganisms, offering a support for growth especially when it concerns organic aggregates, but also a protected area with limited predation. The presence of particles in aquatic systems is known to stimulate microbial productivity and respiration (Simon 2002, Ghiglione *et al.*2009). However, detailed analyses on sorted plastic particles are scarce. So far, the only study dealing with the bacterial communities living in the so-called 'plastisphere' showed a high diversity composed of heterotrophs, autotrophs, predators, symbionts and also some opportunistic pathogens (Zettler *et al.* 2013). The research on degradability of plastics began in the early 1980s and numerous papers provide information on the microbial biodegradation of a variety of plastics such as polyesters,

polyhydroxybutyrate (PHB), polycaprolactone (PCL), polylactic acid (PLA), polyurethane PUR, polyvinyl alcohol (PVA), nylon, and polyethylene (PE)

The descriptor 10 (D 10) of the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) concerns marine litter. Started in 2011, a technical subgroup on marine litter (TSML) aims to provide scientific and technical background for the implementation of MSFD requirements with regard to D 10. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic wastes has assumed increasing importance in the last few years. Indeed, it is important to consider the microbial degradation of synthetic plastics in order to understand what is necessary for their biodegradation. This requires understanding of the interactions between materials and microorganisms and the biochemical changes involved.The current research on the biodegradation of synthetic plastics by microorganisms. The microbial biodegradation of plastic materials includes several steps that are described here. Biodegradation is not disconnected from abiotic degradation, since several studies about biodegradation of some polymers show that the abiotic degradation (mechanical, light, thermal or chemical degradation) precedes microbial assimilation.

Different steps of plastic degradation by microorganisms

Several steps occur in the plastic biodegradation process (Figure 1) and could be identified by specific terminology

-**Bio-deterioration** defines the action of microbial communities and other decomposer organisms responsible for the physical and chemical deterioration that resulted in a superficial degradation that modifies the mechanical, physical and chemical properties of the plastic.

-**Bio-fragmentation** refers to the catalytic actions that cleave polymeric plastics into oligomers, dimers or monomers by ecto-enzymes or free-radicals secreted by microorganisms.

-**Assimilation** characterizes to the integration of molecules transported in the cytoplasm in the microbial metabolism.

-**Mineralisation** refers to the complete degradation of molecules that resulted in the excretion of completely oxidized metabolites (CO_2, N_2, CH_4, H_2O).

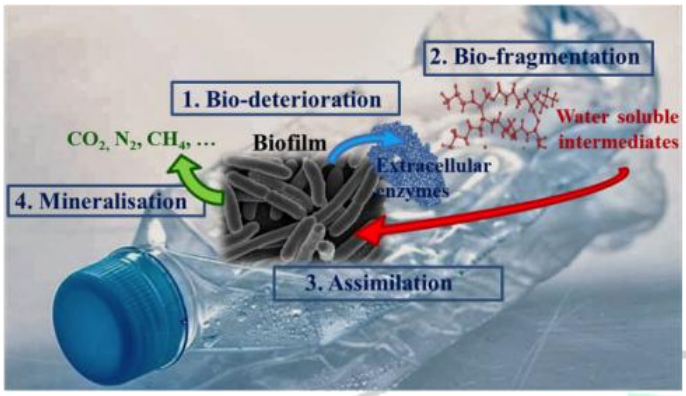

Fig. 1. The different steps of plastic biodegradation by microorganisms

Herein are described the different degrees of the biodegradation process (bio-deterioration, bio-fragmentation, assimilation and mineralisation). The technical estimations adapted to each level of biodegradation are given in Table 1 and Table 2.

Biofertilisers:

Since most of the pulse crops are grown without supply of inorganic nitrogen (chemical) fertilizers, their growth is dependent on the supply of combined nitrogen by nitrogen fixing bacteria. Rhizobial inoculants production involves isolation of efficient strains of Rhizobia, culturing of strains specific for a particular crop in shake flasks or fermentors and mixing of peat. The mixture is allowed to be cured for a short period and then packed in sterile polythene bags. The packets are then used for inoculating the seeds before sowing. *Azotobactor* and *Azospirillum* inoculants are also produced on the same principle and used for non-leguminous crops.

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