BM E52 - ELEMENTS OF BIOTECHNOLOGY

UNIT –I

2 MARKS

1. **Define Biotechnology. (NOV 2014, SEP 2020)**
   - ❖ The science of the production processes based on the action of microorganisms and their active components and of production processes involving the use of cells and tissues from higher organisms.
   - ❖ The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services.
2. **Write a note on Whittaker's five kingdom classification of microorganism. (NOV 2014)**
   - ❖ Monera
   - ❖ Protista
   - ❖ Fungi
   - ❖ Plantae
   - ❖ Animalia
3. **What are the functions of nucleus? (NOV 2015)**
   - ❖ The nucleus is responsible for storage as well as the transfer of genetic materials in the form of DNA or RNA.
   - ❖ It aids in the process of transcription by the synthesis of mRNA molecules.
4. **How microorganisms are classified. (NOV 2015, SEP 2020)**

Microorganisms are classified based on physiological and morphological characteristics such as

- ❖ Bacteria
- ❖ Fungi
- ❖ Virus
- ❖ Algae
- ❖ Protozoa

5. **Write a short note on Mitochondria. (APRIL\MAY 2016)**
   - ❖ Mitochondria are double membrane-bound cell organelles responsible for the supply and storage of energy for the cell.
   - ❖ The primary purpose of mitochondria is the oxidation of various substrates in the cell to release energy in the form of ATP (Adenosine Triphosphate).
   - ❖ So it is called as respiration part of cells.

6. Discuss the difference between plant cell and animal cell. (APRIL\MAY 2016)

Plant cell	Animal cell
Plant cell are larger than animal cell.	Animal cell are generally small in size.
The plasma membrane of plant cell is surrounded by a rigid cell wall.	Cell wall is absent.
Plastids are present	Except the protozoan euglena no animal cell possesses plastids.
Most mature plant cells have large Center vacuole.	Vacuoles in animal cell are many and small.
Plant cell lack centrosomes and centrioles.	Animal cell have centrosomes and centrioles.

7. Biotechnology is an interdisciplinary science. Justify it. (MAY 2017)

- Biotechnology is prior an interdisciplinary pursuit.
- Interdisciplinary application occurs when the blending of ideas that occur during multidisciplinary cooperation leads to the crystallisation of a new disciplinary area with its own concepts and methodologies.

8. Mention the limitations of biotechnology.(MAY 2017)

- Genetically engineered food may cause health problems (Bt. cotton, brinjal, corn etc. have a toxic gene of bacterium)
- Pests resistant Bt plants also kill useful insects like butterfly, bees along with disease causing pest insect ecological imbalance.
- Poor regulation.
- High cost of development.

9. Comment on the public perception of biotechnology. (NOV\DEC 2017)

Public perception of biotechnology will have a major influence on the rate and direction of developments and there is growing concern about genetically modified products associated with genetic manipulation are diverse questions of safety, ethics and welfare.

10. Mention the types of RNA and their functions.(NOV\DEC 2017)

There are three main types of RNA, all involved in protein synthesis.

- ❖ Messenger RNA (mRNA) serves as the intermediary between DNA and the synthesis of protein products during translation.
- ❖ Ribosomal RNA (rRNA) is a type of stable RNA that is a major constituent of ribosomes.
- ❖ Transfer RNA (tRNA) is a small type of stable RNA that carries an amino acid to the corresponding site of protein synthesis in the ribosome.

11. Define a cell. (APRIL\MAY 2018)

- ❖ The cell is the smallest structural and functional unit of an organism, which is seen by typically microscopic and consists of cytoplasm and a nucleus enclosed in a membrane.
- ❖ These cells cooperate with other specialized cells and become the building blocks of large multicellular organisms, such as humans and other animals.

12. Enumerate the difference between DNA and RNA. (APRIL\MAY 2019)

DNA	RNA
Sugar is deoxyribose	Sugar is ribose
Pyrimidine is equal to purines.	Pyrimidines are not equal to purines.
Not destroyed by alkali.	Destroyed by alkali.
Usually double stranded.	Usually single stranded.
A, B, Z are the different forms of DNA.	t-RNA, r-RNA, m-RNA are the different types of RNA.

13. What are the functions of DNA? (NOV/DEC 2019)

The functions of DNA include

- ❖ Replication
- ❖ Gene expression
- ❖ Mutation
- ❖ Transcription
- ❖ Translation

14. What is genetic material? (MAY 2019)

- Any material of plant, animal, microbial or other origin that carries genetic information and that passes it passes from one generation to other
- DNA or the Deoxyribonucleic acid is the hereditary materials in humans.

11 MARKS

1. **Explain in detail about cell and its organelles with suitable diagram. (NOV 2014, NOV 2015, NOV 2016, NOV 2017, MAY 2019, SEP 2020)**

CELL:

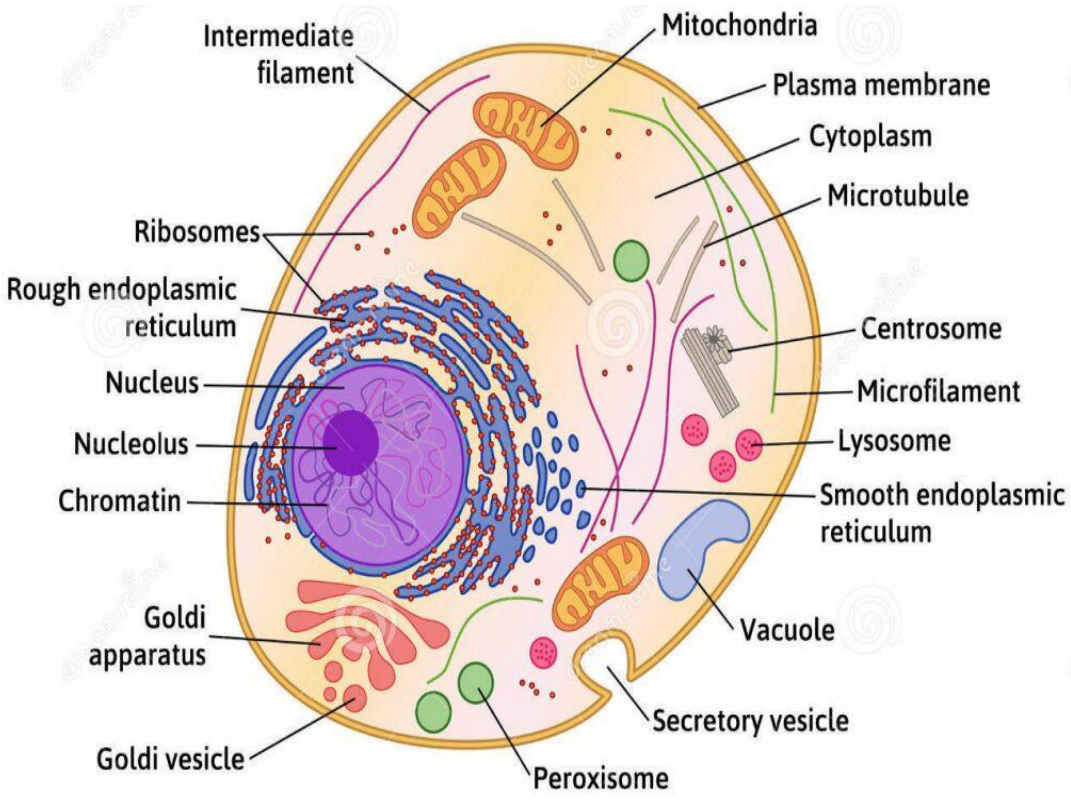
- The cell is the smallest structural and functional unit of an organism, which is seen by typically microscopic and consists of cytoplasm and a nucleus enclosed in a membrane.
- These cells cooperate with other specialized cells and become the building blocks of large multicellular organisms, such as humans and other animals.

TYPES OF CELL:

Depending upon the nucleus cells are classified into

- **Eukaryotic cell:** It is a type of cell which contains a nucleus; eukaryotes can be either single-celled or multicellular.
- **Prokaryotic cell:** It is a type of cell which does not contain any nucleus. (i.e. unorganized nucleus). That means the genetic material is mixed with cytoplasm.

STRUCTURE OF CELL:



- The cell structure comprises individual components with specific functions essential to carry out life's processes.
- These components include- cell wall, cell membrane, cytoplasm, nucleus, and cell organelles.

FUNCTIONS OF CELL:

- It can provide structure for the body taking nutrients from food and convert those nutrients into energy and carry out specialized functions.
- Cells also contain the body's hereditary material and can make copies of themselves.
- Cells have many parts, each with a different function.

CELL AND ITS ORGANELLES:

- Organelles are also known as cell compartments.
- Eukaryotic cells have membrane bound compartments in which specific metabolic activities takes place.
- The cells of eukaryotic organelles are structurally complex and have organized interior compartments that are enclosed by lipid membranes.

ORGANELLES:

- Nucleus
- Cytoplasm
- Mitochondria
- Ribosome
- Golgi apparatus
- Lysosome
- Endoplasmic reticulum (smooth, rough)
- Peroxisome

NUCLEUS:

- The nucleus is a double membrane-bound structure responsible for controlling all cellular activities as well as a centre for genetic materials, and its transferring.
- It is one of the large cell organelles occupying 10% of total space in the cell.

- It is often termed the “brain of the cell” as it provides commands for the proper functioning of other cell organelles.

STRUCTURE:

- Nucleus consists of a nuclear envelope, nucleolus and chromosomes(chromatin).
- The nuclear envelope is similar to the cell membrane in structure and composition.
- It has pores that allow the movement of proteins and RNA in and outside the nucleus.
- The chromatin in the nucleus contains RNA or DNA along with nuclear proteins, as genetic material that is responsible for carrying the genetic information from one generation to another.
- The nucleolus is like a nucleus within the nucleus.
- It is a membrane-less organelle that is responsible for the synthesis of rRNA and assembly of ribosomes required for protein synthesis.

FUNCTIONS:

- The nucleus is responsible for storage as well as the transfer of genetic materials in the form of DNA or RNA.
- It aids in the process of transcription by the synthesis of mRNA molecules.
- The nucleus controls the activity of all other organelleswhile facilitating processes like cell growth, cell division and synthesis of proteins.

CYTOPLASM:

- Cytoplasm refers to everything present inside the cell except the nucleus.
- That means all the organelles of cell are floating in the liquid inside the cell membrane which is called cytoplasm.
- This liquid is composed of water, ions, minerals and nutrients.

STRUCTURE:

- The cytoplasm consists of a cytosol, a gel-like substance that contains other organelles and insoluble molecules that store energy and are not surrounded by any layer.
- The cytoplasm is colourless and has about 80% water alongwith various nutrients required for the cell.

- Cytoplasm helps in the movement of materials inside the cell by a process termed cytoplasmic streaming.

FUNCTIONS:

- Most of the vital cellular and enzymatic reactions like cellular respiration and translation of mRNA into proteins occur in the cytoplasm.
- It acts as a buffer and protects genetic materials as well as other organelles from damage due to collision or change in the pH of the cytosol.
- It helps in the distribution of various nutrients and doing the movement of cell organelles within the cell.

MITOCHONDRIA:

- Mitochondria are double membrane-bound cell organellesresponsible for the supply and storage of energy for the cell.
- The primary purpose of mitochondria is the oxidation of various substrates in the cell to release energy in the form of ATP (Adenosine Triphosphate).
- So it is called as respiration part of cells.
- It is 150times smaller in size compared with nucleus.

FUNCTIONS:

- A mitochondrion contains two membranes with the outer layer being smooth while the inner layer is marked with folding and finger-like structure.
- Mitochondria are also home to single or double-stranded DNA called mDNA that is capable of producing 10% of the proteins present in the mitochondria.
- The primary function of mitochondria is the synthesis of energy in the form of ATP required for the proper functioning of all the cell organelles.
- Mitochondria also help in balancing the amount of Ca+ ions within the cell.
- Different segments of hormones and components of blood are built within mitochondria.
- It has ability to detoxify ammonia in liver.

RIBOSOMES:

- Ribosomes are the site of biological protein synthesis in all living organisms.

- They arrange the amino acids in the order indicated by tRNA and assist in protein synthesis.

LYSOSMES:

- Lysosome has occurred in the cytoplasm of animal cells.
- These organelles contain an array of hydrolytic enzymes(glucanase, protease, cellulose) required for the degradation of various macromolecules. (fat & carbs)
    - **Primary lysosome-** contains hydrolytic enzymes like lipases, amylases, proteases, and nucleases.
    - **Secondary lysosome-** formed by the fusion of primary lysozymes containing engulfed molecules

STRUCTURE:

- The shape of lysozymes is irregular or pleomorphic; however, mostly, they are found in the spherical or granular structure.
- Lysozymes are surrounded by a lysosomal membrane that contains the enzymes within the lysosome and protects the cytosol with the rest of the cell from the harmful action of the enzymes.

FUNCTIONS:

- It is responsible for intracellular digestion where the larger macromolecules are degraded into smaller molecules with the help of enzymes present in them.
- Lysozymes also perform the critical function of the autolysis of unwanted organelles within the cytoplasm.
- Besides these, the lysosome is involved in various cellular processes, including secretion, plasma membrane repair, cell signalling, and energy metabolism.

GOLGI APPARATUS:

- The Golgi apparatus is responsible for the packaging and distribution of macromolecules so that they can be sent out to their site of action or other cells.
- Materials (proteins, fats, nutrients, enzymes) produced by ribosomes is transmitted to Golgi bodies by the help of endoplasmic reticulum.
- After some modification or alteration done in Golgi bodies, then it is send to lysosomes.

STRUCTURE:

- The structure of the Golgi bodies is typically exists in three forms, i.e. cisternae, vesicles, and tubules.
- **The cisternae**, which is the smallest unit, has a flattened sac-like structure which is arranged in bundles.
- **Tubules** are present as tubular and branched structures thatradiate from the cisternae.
- **Vesicles** are spherical bodies.

ENDOPLASMIC RETICULUM:

- Endoplasmic Reticulum (ER) is present as an interconnection of tubes that are connected to the nuclear membrane (nucleus) in eukaryotic cells.
- It is used for the function of transportation of particular substances from one cell to another cell.
- It provides space to stay or living of ribosomes.
- Ribosomes – preparation or synthetization of particular substances (proteins, fats. etc.,)
- There are two types of ER based on the presence or absence of ribosomes on them:

STRUCTURE:

Rough ER (RER) - which ribosomes attached on the cytosolic face of Endoplasmic Reticulum and thus are involved in protein synthesis.

Smooth ER (SER) - which lacks ribosomes (i.e. absence of ribosomes) and has a function during lipid (fat) synthesis.

- Endoplasmic Reticulum exists in three forms viz. cisternae, vesicles, and tubules.

Cisternae are sac-like flattened, unbranched structures that remain stacked one on top of another.

Vesicles are spherical structures that carry proteins throughout the cell.

Tubules are tubular branched structures forming a connection between cisternae and vesicles.

PEROXISOMES:

- Peroxisomes are oxidative membrane-bound organelles found in the cytoplasm of all eukaryotes.
- The name is accredited due to their hydrogen peroxide generating and removing activities.

STRUCTURE:

- Peroxisome consists of a single membrane and granular matrix scattered in the cytoplasm.
- They exist either in the form of interconnected tubules or as individual peroxisomes.
- The compartments within every peroxisome allow the creation of optimized conditions for different metabolic activities. (Chemical reactions to produce energy).

FUNCTIONS:

- Peroxisomes are involved in the production and elimination of hydrogen peroxide during biochemical processes.
- Oxidation of fatty acids takes place within peroxisomes.

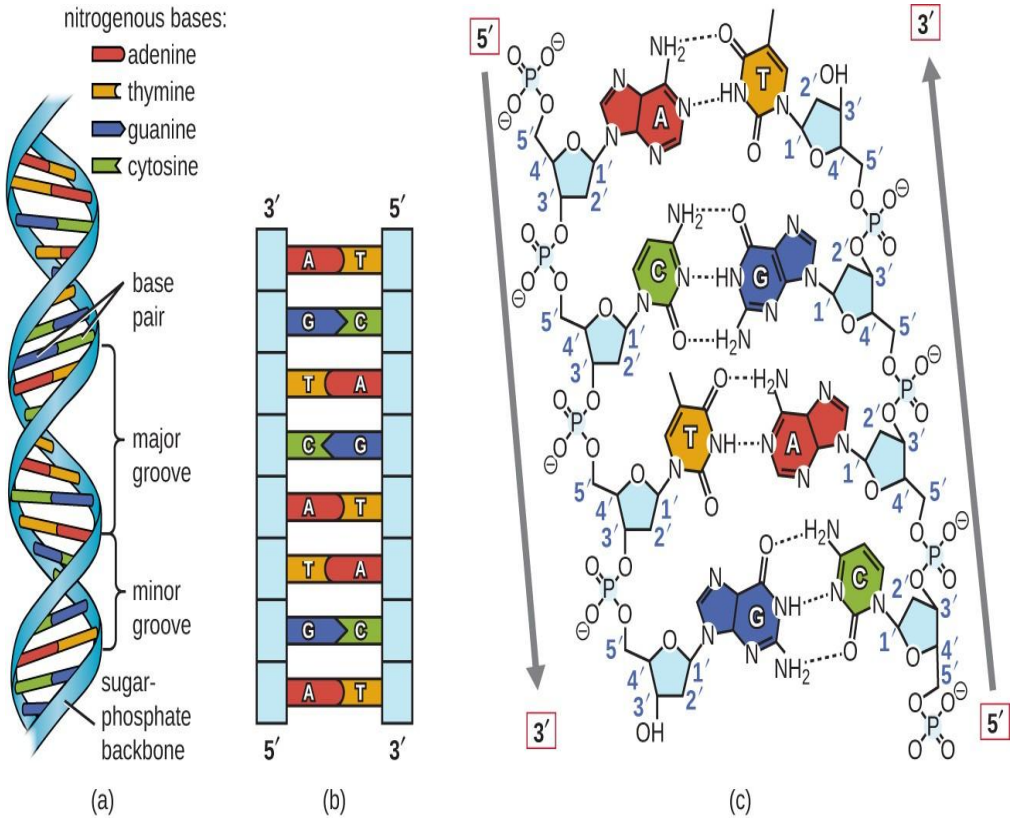
  Additionally, peroxisomes are also involved in the synthesis of lipid(fat)-like cholesterol and plasminogen.

2. Describe in detail about Watson and Crick model of DNA helix.(NOV 2014, MAY 2017, MAY 2018)

STRUCTURE:

- Watson and Crick proposed that DNA is made up of two strands that are twisted around each other to form a right-handed helix.
- The two DNA strands are antiparallel, such that the 3′ end of one strand faces the 5′ end of the other. The 3′ end of each strand has a free hydroxyl group, while the 5′ end of each strand has a free phosphate group.
- The sugar and phosphate of the polymerized nucleotides form the backbone of the structure, whereas the nitrogenous bases are stacked inside.
- These nitrogenous bases on the interior of the molecule interact with each other, base pairing. Analysis of the diffraction patterns of DNA has determined that there are approximately 10 bases per turn in DNA.
- The asymmetrical spacing of the sugar-phosphate backbones generates major grooves (where the backbone is far apart) and minor grooves (where the backbone is close together)
- These grooves are locations where proteins can bind to DNA. The binding of these proteins can alter the structure of DNA, regulate replication, or regulate transcription of DNA into RNA.

- Base pairing takes place between a purine and pyrimidine. In DNA, adenine (A) and thymine (T) are complementary base pairs, and cytosine (C) and guanine (G) are also complementary base pairs, explaining Chargaff's rules.
- The base pairs are stabilized by hydrogen bonds; adenine and thymine form two hydrogen bonds between them, whereas cytosine and guanine form three hydrogen bonds between them.
- In the laboratory, exposing the two DNA strands of the double helix to high temperatures or to certain chemicals can break the hydrogen bonds between complementary bases, thus separating the strands into two separate single strands of DNA (single-stranded DNA [ssDNA]).
- This process is called DNA denaturation and is analogous to protein denaturation, as described in Proteins.
- The ssDNA strands can also be put back together as double-stranded DNA (dsDNA), through reannealing or renaturing by cooling or removing the chemical denaturants, allowing these hydrogen bonds to reform.
- The ability to artificially manipulate DNA in this way is the basis for several important techniques in biotechnology.
- Because of the additional hydrogen bonding between the C = G base pair, DNA with a high GC content is more difficult to denature than DNA with a lower GC content.



FUNCTIONS:

- DNA stores the information needed to build and control the cell. The transmission of this information from mother to daughter cells is called vertical gene transfer and it occurs through the process of DNA replication.
- DNA is replicated when a cell makes a duplicate copy of its DNA, then the cell divides, resulting in the correct distribution of one DNA copy to each resulting cell.
- DNA can also be enzymatically degraded and used as a source of nucleosides and nucleotides for the cell. Unlike other macromolecules, DNA does not serve a structural role in cells.

TYPES:

There are three different DNA types:

A-DNA- It is a right-handed double helix similar to the B-DNA form. Dehydrated DNA takes an A form that protects the DNA during extreme condition such as desiccation. Protein binding also removes the solvent from DNA and the DNA takes an A form.

B-DNA- This is the most common DNA conformation and is a right-handed helix. Majority of DNA has a B type conformation under normal physiological conditions.

Z-DNA- Z-DNA is a left-handed DNA where the double helix winds to the left in a zig-zag pattern. It was discovered by Andres Wang and Alexander Rich. It is found ahead of the start site of a gene and hence is believed to play some role in the gene regulation.

DNA REPLICATION:

DNA replication is an important process that occurs during cell division. It is also known as semi-conservative replication, during which DNA makes a copy of itself.

DNA replication takes place in three stages:

Step 1: Initiation

The replication of DNA begins at a point known as the origin of replication. The two DNA strands are separated by the DNA helicase. This forms the replication fork.

Step 2: Elongation

DNA polymerase III reads the nucleotides on the template strand and makes a new strand by adding complementary nucleotides one after the other. For e.g., if it reads an Adenine on the template strand, it will add a Thymine on the complementary strand.While adding nucleotides to

the lagging strand, gaps are formed between the strands. These gaps are known as Okazaki fragments. These gaps or nicks are sealed by ligase.

Step 3: Termination

The termination sequence present opposite to the origin of replication terminates the replication process. The TUS protein (terminus utilization substance) terminator sequence pauses the leading fork until the lagging strand arrives and induces termination.

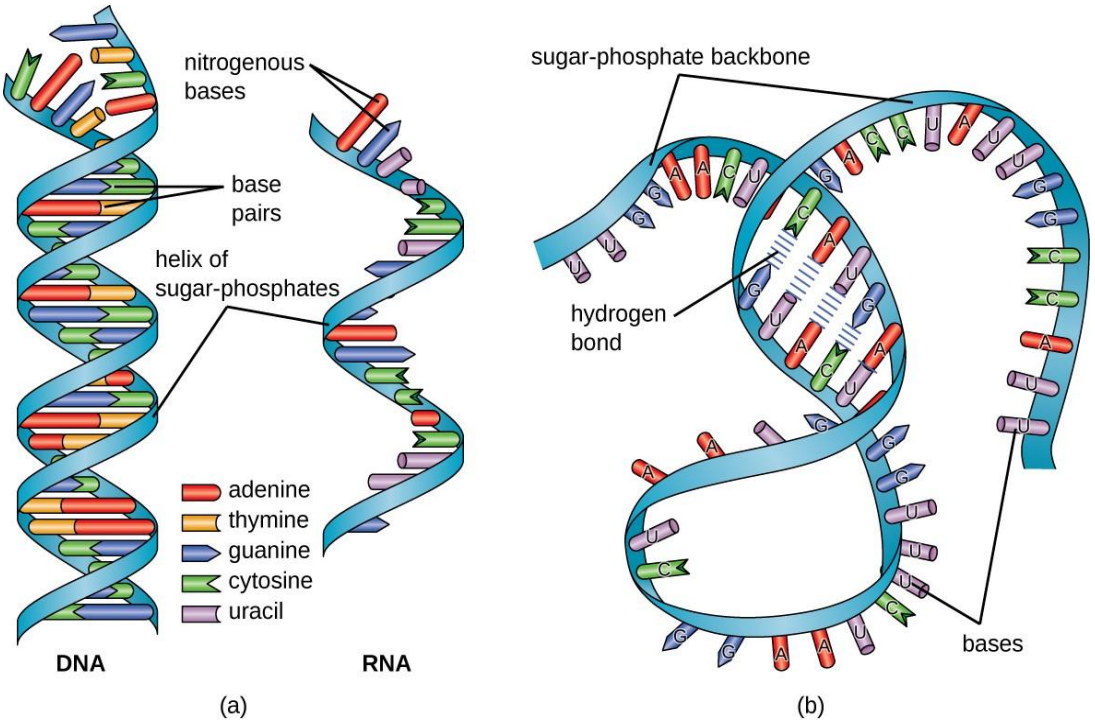
DNA FUNCTION:

- Replication process: Transferring the genetic information from one cell to its daughters and from one generation to the next
- Equal distribution of DNA during the cell division
- Mutations: The changes which occur during the DNA sequences
- Transcription
- Cellular Metabolism
- DNA Fingerprinting
- Gene Therapy

3. Explain the structure and functionof different types of RNA. (NOV 2015, MAY 2016, NOV 2016, NOV 2018, SEP 2020)

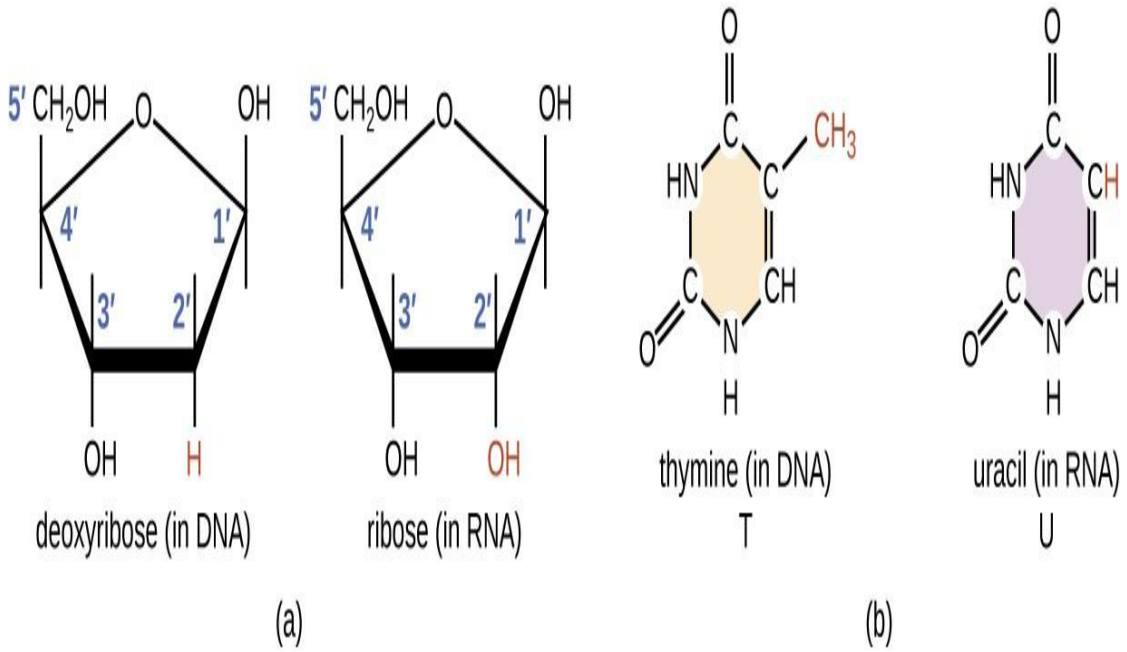
STRUCTURE:

- RNA is typically single stranded and is made of ribonucleotides that are linked by phosphodiester bonds.
- A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases (A, U, G, and C), and a phosphate group.
- The subtle structural difference between the sugars gives DNA added stability, making DNA more suitable for storage of genetic information, whereas the relative instability of RNA makes it more suitable for its more short-term functions.
- The RNA-specific pyrimidine uracil forms a complementary base pair with adenine and is used instead of the thymine used in DNA. Even though RNA is single stranded, most types of RNA molecules show extensive intramolecular base pairing between complementary sequences within the



Functions of RNA in Protein Synthesis

- Cells access the information stored in DNA by creating RNA to direct the synthesis of proteins through the process of translation.
- Proteins within a cell have many functions, including building cellular structures and serving as enzyme catalysts for cellular chemical reactions that give cells their specific characteristics.
- The three main types of RNA directly involved in protein synthesis are messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA).
- Evidence supporting their hypothesis was gathered soon afterwards showing that information from DNA istransmitted to the ribosome for protein synthesis using mRNA.
- If DNA serves as the complete library of cellular information, mRNA serves as a photocopy of specific information needed at a particular point in time that serves as the instructions to make a protein.
- The mRNA carries the message from the DNA, which controls all of the cellular activities in a cell.
- If a cell requires a certain protein to be synthesized, the gene for this product is “turned on” and the mRNA is synthesized through the process of **transcription**.
- The mRNA then interacts with **ribosomes** and other cellular machinery to direct the synthesis of the protein it encodes during the process of **translation**.
- mRNA is relatively unstable and short-lived in the cell, especially in prokaryotic cells, ensuring that proteins are only made when needed.



- In prokaryotes and eukaryotes, tRNA and rRNA are encoded in the DNA, then copied into long RNA molecules that are cut to release smaller fragments containing the individual mature RNA species.
- In eukaryotes, synthesis, cutting, and assembly of rRNA into ribosomes takes place in the nucleolus region of the nucleus, but these activities occur in the cytoplasm of prokaryotes.
- Neither of these types of RNA carries instructions to direct the synthesis of a polypeptide, but they play other important roles in protein synthesis.
- Ribosomes are composed of rRNA and protein. As its name suggests, rRNA is a major constituent of **ribosomes**, composing up to about 60% of the ribosome by mass and providing the location where the mRNA binds.
- The rRNA ensures the proper alignment of the mRNA, tRNA, and the ribosomes; the rRNA of the ribosome also has an enzymatic activity (**peptidyl transferase**) and catalyses the formation of the peptide bonds between two aligned amino acids during protein synthesis.
- Transfer RNA is the third main type of RNA and one of the smallest, usually only 70–90 nucleotides long.
- It carries the correct amino acid to the site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized.
- Any mutations in the tRNA or rRNA can result in global problems for the cell because both are necessary for proper protein synthesis.

Structure and Function of RNA:

	mRNA	rRNA	tRNA
Structure	Short, unstable, single-stranded RNA corresponding to a gene encoded within DNA.	Longer, stable RNA molecules composing 60% of ribosome's mass.	Short (70-90 nucleotides), stable RNA with extensive intramolecular base pairing; contains an amino acid binding site and an mRNA binding site.
Function	Serves as intermediary between DNA and protein; used by ribosome to direct synthesis of protein it encodes.	Ensures the proper alignment of mRNA, tRNA, and ribosome during protein synthesis; catalyzes peptide bond formation between amino acids.	Carries the correct amino acid to the site of protein synthesis in the ribosome.

4. Discuss the classification of microorganisms. (MAY 2016, MAY 2018, NOV 2018)

- Microorganisms are divided into seven types: bacteria, archaea, protozoa, algae, fungi, viruses, and multicellular animal parasites (helminths).
- Each type has a characteristic cellular composition, morphology, mean of locomotion, and reproduction.
- Microorganisms are beneficial in producing oxygen, decomposing organic material, providing nutrients for plants, and maintaining human health, but some can be pathogenic and cause diseases in plants and humans.
- Microorganisms or microbes are microscopic organisms that exist as unicellular, multicellular, or cell clusters.

- Microorganisms are widespread in nature and are beneficial to life, but some can cause serious harm. They can be divided into six major types: bacteria, archaea, fungi, protozoa, algae, and viruses.

BACTERIA:

- Bacteria are unicellular organisms. The cells are described as prokaryotic because they lack a nucleus.
- They exist in four major shapes: bacillus (rod shape), coccus (spherical shape), spirilla (spiral shape), and vibrio (curved shape).
- Most bacteria have a peptidoglycan cell wall; they divide by binary fission; and they may possess flagella for motility. The difference in their cell wall structure is a major feature used in classifying these organisms.
- According to the way their cell wall structure stains, bacteria can be classified as either Gram-positive or Gram-negative when using the Gram staining.
- Bacteria can be further divided based on their response to gaseous oxygen into the following groups: aerobic (living in the presence of oxygen), anaerobic (living without oxygen), and facultative anaerobes (can live in both environments).
- According to the way they obtain energy, bacteria are classified as heterotrophs or autotrophs. Autotrophs make their own food by using the energy of sunlight or chemical reactions, in which case they are called chemoautotrophs.
- Heterotrophs obtain their energy by consuming other organisms. Bacteria that use decaying life forms as a source of energy are called saprophytes.

ARCHAEA:

- Archaea or Archaebacteria differ from true bacteria in their cell wall structure and lack peptidoglycans. They are prokaryotic cells with avidity to extreme environmental conditions.
- Based on their habitat, all Archaeans can be divided into the following groups: methanogens (methane-producing organisms), halophiles (archaeans that live in salty environments), thermophiles (archaeans that live at extremely hot temperatures), and psychrophiles (cold-temperature Archaeans).
- Archaeans use different energy sources like hydrogen gas, carbon dioxide, and sulphur. Some of them use sunlight to make energy, but not the same way plants do.
- They absorb sunlight using their membrane pigment, bacteriorhodopsin. This reacts with light, leading to the formation of the energy molecule adenosine triphosphate (ATP).

FUNGI:

- Fungi (mushroom, molds, and yeasts) are eukaryotic cells (with a true nucleus). Most fungi are multicellular and their cell wall is composed of chitin.
- They obtain nutrients by absorbing organic material from their environment (decomposers), through symbiotic relationships with plants (symbionts), or harmful relationships with a host (parasites).
- They form characteristic filamentous tubes called hyphae that help absorb material. The collection of hyphae is called mycelium. Fungi reproduce by releasing spores.

PROTOZOA:

- Protozoa are unicellular aerobic eukaryotes. They have a nucleus, complex organelles, and obtain nourishment by absorption or ingestion through specialized structures.
- They make up the largest group of organisms in the world in terms of numbers, biomass, and diversity. Their cell walls are made up of cellulose.
- Protozoa have been traditionally divided based ontheir mode of locomotion: flagellates produce their own food and use their whip-like structure to propel forward, ciliates have tiny hair that beat to produce movement, amoeboid have false feet or pseudopodia used for feeding and locomotion, and protozoans are non-motile.
- They also have different means of nutrition, which groups them as autotrophs or heterotrophs.

ALGAE:

- Algae, also called cyanobacteria or blue-green algae, are unicellular or multicellular eukaryotes that obtain nourishment by photosynthesis.
- They live in water, damp soil, and rocks and produce oxygen and carbohydrates used by other organisms. It is believed that cyanobacteria are the origins of green land plants.

VIRUSES:

- Viruses are no cellular entities that consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat. Although viruses are classified as microorganisms, they are not considered living organisms. Viruses cannot reproduce outside a host cell and cannot metabolize on their own. Viruses often infest prokaryotic and eukaryotic cells causing diseases.

MULTICELLULAR ANIMAL PARASITES:

- A group of eukaryotic organisms consisting of the flatworms and roundworms, which are collectively referred to as the helminths.
- Although they are not microorganisms by definition, since they are large enough to be easily seen with the naked eye, they live a part of their life cycle in microscopic form.
- Since the parasitic helminths are of clinical importance, they are often discussed along with the other groups of microbes.

5. Explain the importance of scope of biotechnology. (MAY 2017, NOV 2017)

- Biotechnology is the technologies applied to biology, molecular biology, genetics, and many other subfields of biology. Biotechnology utilizes cellular and bimolecular processes to create technologies and products that help improve our lives and the nature.
- By making useful food, such as bread and cheese, and preserving dairy products, we have done these for many years by now.
- Recent biotechnology develops breakthrough products and technologies to fight diseases, reduce our environmental harm, feed the hungry, use less and cleaner energy, and have safer, cleaner and more efficient industrial manufacturing processes.

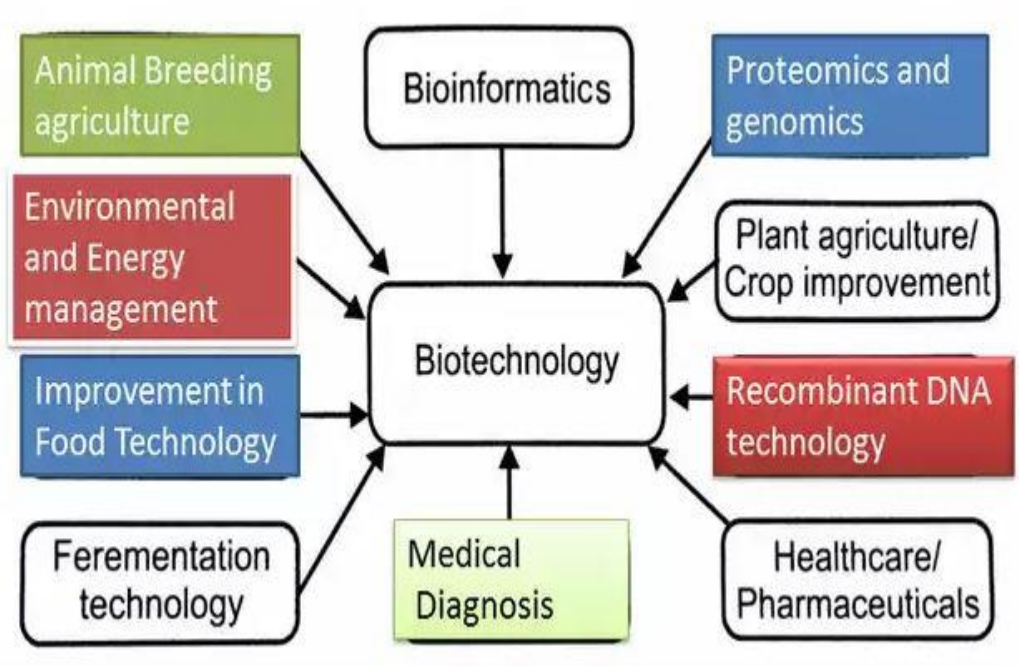


Fig.1 Scope of Biotechnology in various fields

- So far, more than 250 biotechnology health care products and vaccines have been made available to patients, many for previously untreatable diseases.
- More than 13.3 million farmers around the world use agricultural biotechnology to increase yields, prevent damage from insects and pests and reduce damage done on environment due to farming.
- And more than 50 bio refineries are being built across North America to test and refine technologies to produce bio fuels and chemicals from renewable biomass, which can help reduce greenhouse gas emissions.

SCOPE IN MEDICINE:

- In medicine Biotechnology has genetic engineering that motivated expectations for drugs, therapeutic proteins, and various biological organisms. It includes engineering yeasts, pesticides, seeds and also modified human cells. The modified cells are used to treat multiple genetic diseases.
- The genetically-modified food, cloning, stem cell research and gene therapy are the most significant benefits of genetic engineering that make this field an essential one in the modern world. There is a broad range of biotechnology products available for therapeutic use.
- The biotechnology-derived medicines are derived from different kinds of expression systems like a plant or insect cells, transgenic animals, mammalian, yeast, Escherichia coli and more. This kind of expressed gene or protein includes the same nucleotide sequence or amino acid as the endogenous form of human.
- The primary function of these products is to prevent and treat various human diseases.
- **MOLECULAR DIAGNOSIS:** It is one of the most popular and helpful biotechnology applications used in this immediate health care filed. People who have the symptoms created by pathogens are readily known to be diseases. Most of the individuals fail to diagnose this condition earlier, so they will get lots of problems.
- **MEDICAL BIOTECHNOLOGY:** The medical biotechnology is another important application of Biotechnology in medicine. As an active application, it generally deals with proper use of the recombinant DNA technology in different therapeutic processes.
- E. coli is the leading resource of this genetically engineered insulin. It is produced with an aim to compatible with the human body. Moreover, it is manufacturing efficiently to deliver positive results.
- It is one of the heredity techniques used for ending various genetic diseases and other genetic related problems in carrier screening, parents, and sex. It is the best technique that uses suitable DNA probes that have a better sequence similar to mutated sequences.

SCOPE IN AGRICULTURE:

- Agricultural biotechnology, also known as Agritech, is an area of agricultural science involving the use of scientific tools and techniques, including genetic engineering, molecular markers, molecular diagnostics, vaccines, and tissue culture, to modify living organisms: plants, animals, and microorganisms.
- Crossbreeding mates' two sexually compatible species to create a new and special variety with the desired traits of the parents. For example, the honey crisp apple exhibits a specific texture and flavour due to the crossbreeding of its parents.
- Protoplasmic fusion is the joining of cells or cell components to transfer traits between species. For example, the trait of male sterility is transferred from radishes to red cabbages by protoplast fusion. This male sterility helps plant breeders make hybrid crops.
- Biotechnology allows farmers to grow more food on less land using farming practices that are environmentally sustainable.
- Seeds yield more per acre, plants naturally resist specific insect pests and diseases, and farming techniques improve soil conservation.
- Farmers and ranchers can help plants and animals fight diseases and adapt to environmental stress and climate change. We can enhance the nutritional content of foods and improve human health through plant- and animal-produced therapies.
- The primary biotech crops grown in the United States are corn, cotton, and soybeans, but also canola, squash, papaya, alfalfa, and sugar beet.
- Additionally, crops can be engineered to reduce toxicity or to produce varieties with removed allergens.

SCOPE IN INDUSTRY:

- The scope of Industrial Biotechnology course is to give opportunities to make a change in the field of development. The field of Industrial Biotechnology is gaining a lot of support from the Government and is also funding to allow the companies in developing the processes that are involved in the change.
- Industrial biotechnology is one of the most promising new approaches to pollution prevention, resource conservation, and cost reduction.
- It is often referred to as the third wave in biotechnology.
- The application of biotechnology to industrial processes is not only transforming how we manufacture products but is also providing us with new products that could not even be

imagined a few years ago. Because industrial biotechnology is so new, its benefits are still not well known or understood by industry, policymakers, or consumers.

- Biotechnology companies developed enzymes that removed stains from clothing better than phosphates, thus enabling replacement of a polluting material with a non-polluting bio based additive while improving the performance of the end product.
- This innovation dramatically reduced phosphate-related algal blooms in surface waters around the globe, and simultaneously enabled consumers to get their clothes cleaner with lower wash water temperatures and concomitant energy savings.
- It is expected that industrial biotechnology will be increasingly adopted by chemical, pharmaceutical, food, and agricultural industries.

6. Explain in detail about the public perception of biotechnology. (MAY 2019)

- Biotechnology is the use or development of techniques using organisms (or parts of organisms) to provide or improve goods or services.
- Genetic engineering is one part of biotechnology and involves design of the DNA or an organism.
- Genetic engineering is also often perceived as a technology associated with many risks, and most people have both these feelings about it.
- It is important to see these benefits and risks in an international way because the world is becoming smaller and ever more interdependent.
- All people of the world can benefit if they can access medicines, and more environmentally sustainable agriculture.
- However, biotechnological inventions that allow industrialized countries to become self-sufficient in many products change the international trade balances and prosperity of people in and between developing and industrialized countries.
- If developing countries cannot export products because of product substitution the result may be political instability and war.
- Example, the use of enzymatic conversion of corn starch into high fructose corn syrup causes serious damage to the economies of sugar exporting nations, and may already have caused political instability in some.
- **Perceptions of public 1-** Consumer awareness, perceptions and attitudes have played and will play a huge role in the acceptance of GM technologies. To understand the awareness, opinions and risk perception of consumers/ common people regarding GM foods and crops a survey was conducted of a sample of urban residents/ consumers in the four sample states.

- **Perceptions of public 2-** The data below describes the sample profile and shows that the respondents belong to the four states: Andhra Pradesh, Gujarat, Maharashtra and Punjab. Majority of the respondents belong to the middle-age group of 31 to 50, the average age being 37 years.
- **Consumer Survey-** Sample profile State Respondents (%) (N= 115) Andhra Pradesh 28.70, Gujarat 19.13, Maharashtra 27.83, Punjab 24.35
- Preliminary questioning showed that familiarity with genetically modified(GM) or genetically engineered (GE) foods/ crops was far from universal – most did not know what it was and had never heard about it.
- Only those who, after some description, could understand at least a little about the subjects were interviewed.
- Only 9.7 percent indicated that they are very familiar, 40.7 percent were somewhat familiar, and 47.8 percent were not very familiar. Thus, the awareness level of the people regarding GM/GE appears to be quite low.
- Whereas 88.7 percent had heard of Bt cotton and 47 percentages about Bt Brinjal, the majority have not heard of other GM crops.
- Familiarity of respondents with GM/GE (%) N= 115 Very familiar somewhere at familiar Not very familiar had never heard the terms.
- Analysis of the sources of information indicates that for 70 percent of people the source of information is newspapers and for 35 percent it is TV & Radio.
- Another important source is Government agencies at 37 percent. Friends & Relatives are indicted by only 15 percent of the respondents, and NGOs by only 3 percent.
- Experts, scientists and teachers come at less than 5 percent. Public debates generally on TV are indicted by 13 percent. Thus, newspapers and TV-Radio dominate as the source of the information
- Regarding the awareness level, only 11 percent strongly agree that they are well aware and 37 percent only partially agree.
- About 50 percent are unaware that GM foods are allowed in other countries.
- Less than 25 percent have tried to look up scientific or research findings about them.
- The responses indicate that a large majority of the respondent are aware of the potential benefits of GM technology such as higher yields, less pesticides and better quality and nutrition.
- The majority also thinks that they would be cheaper. Many think that the resistance is politically motivated and that GM technology should be supported by the government.

- Many though are not clear about their threat to human life or their great usefulness. But over 80 percent think that the resistance to GM is due to poor awareness and information.
- This indicates that people are broadly aware of the benefits of GM technology but lack clear knowledge and are concerned about the risks.
- It indicates that much can be changed on this front by strong communication and awareness building especially by the government and the experts.

PERCEPTION ABOUT BT COTTON IN INDIA:

- People are aware about the reduction in pesticide use due to BT cotton. Most people are fine with consuming cottonseed oil made of Bt cotton, and wearing cotton clothes made of Bt cotton.
- The findings show that most of the people are aware about the success of Bt cotton in India, and that it has helped increasing cotton productivities dramatically, and improve the economic status of cotton growing farmers. But some are ignorant.
- Consumer perceptions seem to play a major role in the acceptance of GM technologies. Findings show that the familiarity with genetically modified (GM) is far from universal and most people do not know what it was/ had never heard about it.
- Analysis of the sources of information showed that newspapers and TV-Radio dominate as the source of the information.
- Over 50 percent indicate that the public does not have enough information about GM foods/crops.
- Nearly 90 percent agree that people are afraid of GM because of the scare created by the media.
- Over 90 percent agree that negative aspects are amplified by the media and when information moves from person to person, and that media over- estimates the risk of rare events.

BM E52 - ELEMENTS OF BIOTECHNOLOGY

UNIT –II

2 MARKS

1. **List out the properties of Enzymes. (NOV 2014,NOV/DEC 2017)**
   - ❖ Enzymes are biological catalyst.
   - ❖ All enzymes possess active sites which participate in the biochemical reactions.
   - ❖ Enzymes are highly specific in nature
   - ❖ Enzymes increase the rate of reaction and remain unaffected by the reaction which they catalyze.

2. **What are the characteristics of Protein? (NOV 2014)**
   - ❖ Amino acids - proteins are made of long strings of amino acids which are often called the building blocks of life.
   - ❖ Size - when two amino acids come together they form a peptide bond.
   - ❖ Structure - The sequence of amino acids in a protein determines its shape and which is turn into its function.
   - ❖ It can store energy.

3. **What are the advantages of biocatalyst over chemical catalyst? (NOV 2015)**
   - ❖ Biocatalysts can also be used to replace chemical catalysts, and this has the advantage that toxic by-products of chemical catalysis are bypassed.
   - ❖ This makes it cleaner and removes the need to clean up the toxins.
   - ❖ Another advantage of using enzymes is its specificity and ability to function in mild conditions.

4. **What are the types of Enzyme inhibitors? (NOV 2015)**

Enzyme inhibitors are of three types. They are:

- ❖ Competitive inhibitors
- ❖ Non - competitive inhibitors
- ❖ Allostertic inhibitors.

5. **List out the application of protein? (APRIL/MAY 2016)**

- DNA replication
- Responding to stimuli
- Providing structure to cells and organisms
- Catalyzing metabolic reactions

6. What is the function of protein? Or List out the function of protein(APRIL/MAY 2016,MAY 2017)

- Protein are composed of amino acids serve in many role in the body (e.g. as enzymes, structural components, hormones and antibodies)
- Proteins are the molecular instruments through which genetic information is expressed
- They execute their activities in the transport of oxygen and carbon dioxide by the hemoglobin and special enzymes in the red cells.

7. List out the properties of Immobilized enzymes.(NOV 2016)

- Permit the re-use of the component enzyme(s).
- Ideal for continuous operation.
- Product is enzyme free.
- Permit more accurate control of catalytic processes.
- Improve stability of enzymes.
- Allow development of a multi enzyme reaction system.
- Offer considerable potential in industrial and medical use.
- Reduce effluent disposal problems.

8. What is the peptide bond? (NOV 2016)

- A peptide bond is an amide type of covalent chemical bond linking two consecutive alpha-amino acids from C1 (carbon number one) of one alpha-amino acid and N2 (nitrogen number two) of another, along a peptide or protein chain.
- Living organisms use peptide bonds to form long chains of amino acids, known as proteins.

9. Define Enzyme. (MAY 2017)

- An enzyme is a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process.
- Without enzymes, many of these reactions would not take place at a perceptible rate. Enzymes catalyze all aspects of cell metabolism

10. "Enzyme acts as Catalyst "- Justify. (NOV/DEC 2017)

- Enzymes are biological catalysts. Enzymes are proteins functioning as catalysts that speed up reactions by lowering the activation energy.
- It will be present in living organisms which will acts as a catalysts to make chemical changes in molecules.

11. Define a ligand. (APRIL/MAY 2018)

- A ligand is an ion or molecule, which donates a pair of electrons to the central metal atom or ion to form a coordination complex.
- Ligand is usually a molecule which produces a signal by binding to a site on a target protein.
- A ligand can be natural, as an organic or inorganic molecule.

12. What are immobilized enzymes? (APRIL/MAY 2018,NOV/DEC 2018)

- Immobilization of enzymes is a insoluble polymers, such as membranes and particles, which act as supports or carriers for the enzyme activity.
- The enzymes are physically confined during a continuous catalytic process and may be recovered from the reaction mixture and re-used over and over again.

13. Why is the Quaternary structure essential for enzyme action? (NOV/DEC 2018)

- Quaternary structure is an essential component that contributes to the sophisticated allosteric regulation mechanism in a key enzyme from Mycobacterium tuberculosis.
- A cell may conserve valuable resources in the creation of a large protein by repeating the synthesis of a few polypeptide chains many times rather than synthesizing one extremely long polypeptide chain.

14. Describe protein secondary structure? (DEC 2018)

- Secondary structures are formed by a regular repeating pattern of hydrogen bond formation between backbone atoms.
- The secondary structure of protein could be:
  - Alpha-helix
  - Beta-helix

15. Write the application of Enzymes. (Sep 2020)

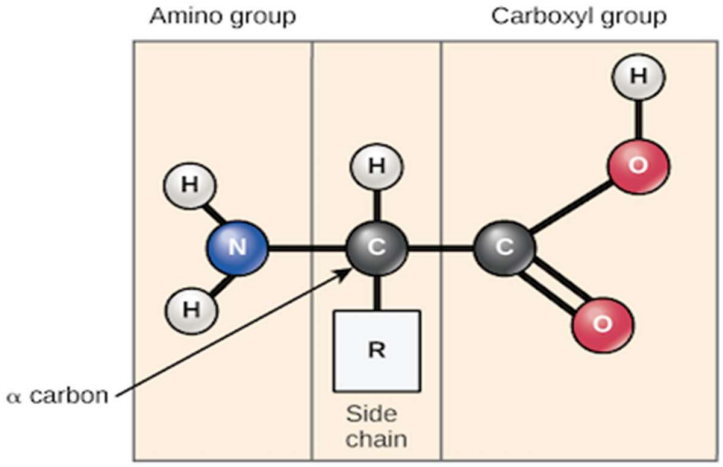
- Starch industry: Convert starch into glucose and various syrups.

- Baking industry: Catalyze breakdown of starch in the flour to sugar, which can be used by the yeast.
- Dairy industry: Manufacture of cheese, used to split protein.
- Textile industry: Traditionally used to treat leather to make it pliable by removing certain protein components.

11 MARKS

1. **Write in detail about structure, functions and properties of proteins. (Nov 2014, May 2016, Nov 2016, May 2019)**

- Proteins are the most abundant biological macromolecules, occurring in all living cells.Proteins are the polymers of amino acids covalently linked by the peptide bonds.
- The building blocks of proteins are the twenty naturally occurring amino acids.



STRUCTURE OF PROTEIN:

- The linear sequence of amino acid residues in a polypeptide chain determines the three-dimensional configuration of a protein, and the structure of a protein determines its function.
- All proteins contain the elements carbon, hydrogen, oxygen, nitrogen and sulfur some of these may also contain phosphorus, iodine, and traces of metals like ion, copper and zinc.
- A protein may contain 20 different kinds of amino acids. Each amino acid has an amine group at one end and an acid group at the other and a distinctive side chain.
- The backbone is the same for all amino acids while the side chain differs from one amino acid to the next.

  The structure of proteins can be divided into four levels of organization:

  - Primary structure

- Secondary structure
- Tertiary structure
- Quaternary structure

Primary structure:

- The primary structure of a protein consists of the amino acid sequence joined by polypeptide bonds.
- Because there are no dissociable protons in peptide bonds, the charges on a polypeptide chain are due only to the N-terminal amino group, the C-terminal carboxyl group, and the side chains on amino acid residues.
- The primary structure determines the further levels of organization of protein molecules.

Secondary structure:

- The secondary structure includes various types of local conformations in which the atoms of the side chains are not involved.
- Secondary structures are formed by a regular repeating pattern of hydrogen bond formation between backbone atoms. The secondary structure involves α-helices, β-sheets.
- The secondary structure of protein could be:
  - Alpha-helix
  - Beta-helix
- The α-helix is a right-handed coiled strand. The side-chain substituent of the amino acid groups in a α-helix extend to the outside.
- Hydrogen bonds form between the oxygen of the C=O of each peptide bond in the strand and the hydrogen of the N-H group of the peptide bond four amino acids below it in the helix.
- The side-chain substituent of the amino acids fit in beside the N-H groups. The hydrogen bonding in a ß-sheet is between strands (inter-strand) rather than within strands (intra-strand).
- The sheet conformation consists of pairs of strands lying side-by-side. The carbonyl oxygen in one strand hydrogen bond with the amino hydrogen of the adjacent strand.
- The two strands can be either parallel or anti-parallel depending on whether the strand directions (N-terminus to C-terminus) are the same or opposite.
- The anti-parallel ß-sheet is more stable due to the more well-aligned hydrogen bonds.

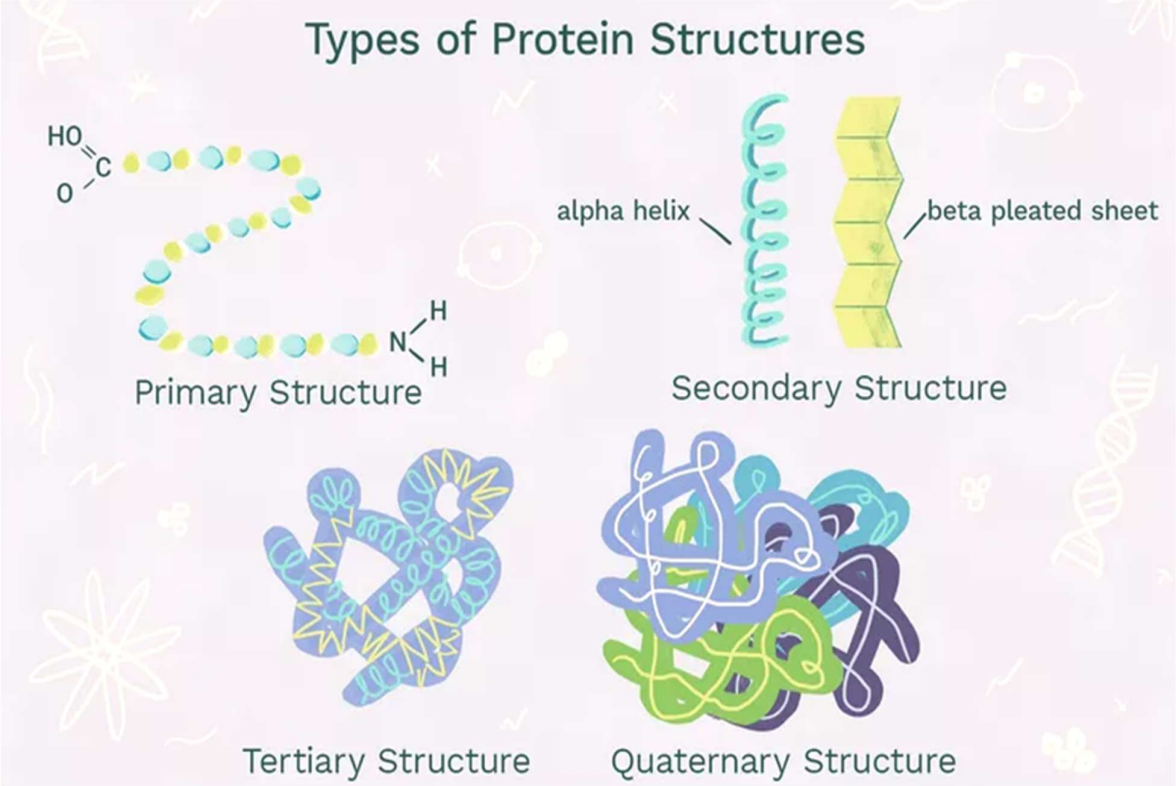
Tertiary structure:

- Tertiary structure of a protein refers to its overall three-dimensional conformation.
- The types of interactions between amino acid residues that produce the three-dimensional shape of a protein include hydrophobic interactions, electrostatic interactions, and hydrogen bonds.

- Covalent disulfide bonds also occur. It is produced by interactions between amino acid residues that may be located at a considerable distance from each other in the primary sequence of the polypeptide chain.
- Hydrophobic amino acid residues tend to collect in the interior of globular proteins, where they exclude water, whereas hydrophilic residues are usually found on the surface, where they interact with water.

Quaternary structure:

- Quaternary structure refers to the interaction of one or more subunits to form a functional protein, using the same forces that stabilize the tertiary structure.
- It is the spatial arrangement of subunits in a protein that consists of more than one polypeptide chain.



FUNCTIONS OF PROTEIN:

- Proteins, which are composed of amino acids, serve in many roles in the body (e.g., as enzymes, structural components, hormones, and antibodies).
- They act as structural components such as keratin of hair and nail, collagen of bone etc.
- Proteins are the molecular instruments through which genetic information is expressed. They execute their activities in the transport of oxygen and carbon dioxide by hemoglobin and special enzymes in the red cells.

- They function in the homo static control of the volume of the circulating blood and that of the interstitial fluids through the plasma proteins. They are involved in blood clotting through thrombin, fibrinogen and other protein factors.
- They act as the defense against infections by means of protein antibodies. They perform hereditary transmission by nucleoproteins of the cell nucleus.
- Ovalbumine, glutelin etc. are storage proteins. Actin, myosin act as contractile protein important for muscle contraction.

PROPERTIES OF PROTEINS:

PHYSICAL PROPERTIES:

- **Colour and Taste**

  Proteins are colourless and usually tasteless. These are homogeneous and crystalline.

- **Shape and Size**

  The proteins range in shape from simple crystalloid spherical structures to long fibrillar structures. Two distinct patterns of shape have been recognized:

  Globular proteins- These are spherical in shape and occur mainly in plants especially in seeds and in leaf cells. These are bundles formed by folding and crumpling of protein chains.

  e.g., pepsin, Edestin, insulin, ribonucleic etc.

  Fibrillar proteins- These are thread-like or ellipsoidal in shape and occur generally in animal muscles. Most of the studies regarding protein structure have been conducted using these proteins. e.g., fibrinogen, myosin etc.

- **Molecular Weight**

  The proteins generally have large molecular weights ranging between 5×103 and 1×106. It might be noted that the values of molecular weights of many proteins lie close to or multiples of 35,000 and 70,000.

- **Colloidal Nature**

  Because of their giant size, the proteins exhibit many colloidal properties, such as; their diffusion rates are extremely slow and they may produce considerable light-scattering in solution, thus resulting in visible turbidity (Tyndall effect).

- **Denaturation**

  Denaturation refers to the changes in the properties of a protein. In other words, it is the loss of biologic activity. In many instances the process of denaturation is followed by coagulation which means a process where denatured protein molecules tend to form large aggregates and to precipitate from solution.

- **Amphoteric Nature**

  Like amino acids, the proteins are amphoteric, i.e., they act as acids and alkalies both. These migrate in an electric field and the direction of migration depends upon the net charge possessed by the molecule. The net charge is influenced by the pH value. Each protein has a fixed value of isoelectric point (pl) at which it will move in an electric field.

- **Ion Binding Capacity**

  The proteins can form salts with both cations and anions based on their net charge.

- **Solubility**

  The solubility of proteins is influenced by pH. Solubility is lowest at isoelectric point and increases with increasing acidity or alkalinity. This is because when the protein molecules exist as either cations or anions, repulsive forces between ions are high, since all the molecules possess excess charges of the same sign. Thus, they will be more soluble than in the isoelectric state.

- **Optical Activity**

  All protein solutions rotate the plane of polarized light to the left, i.e., these are levorotatory.

CHEMICAL PROPERTIES:

- **Hydrolysis**

  Proteins are hydrolyzed by a variety of hydrolytic agents.
  A. By acidic agents: Proteins, upon hydrolysis with conc. HCl (6–12N) at 100–110°C for 6 to 20 hrs, yield amino acids in the form of their hydrochlorides.

  B. By alkaline agents: Proteins may also be hydrolyzed with 2N NaOH.

- **Reactions involving COOH Group**

  A. Reaction with alkalies (Salt formation)

  B. Reaction with alcohols (Esterification)

  C. Reaction with amines

- **Reactions involving NH2 Group**

  A. Reaction with mineral acids (Salt formation): When either free amino acids or proteins are treated with mineral acids like HCl, the acid salts are formed.
  B. Reaction with formaldehyde: With formaldehyde, the hydroxy-methyl derivatives are formed.

  C. Reaction with benzaldehyde: Schiff ‘s bases are formed

D. Reaction with acylating agents (Acylation)

E. Reaction with FDNB or Sanger's reagent.

F. Reaction with dansyl chloride

- **Reactions involving both COOH AND NH2 Group**

A. Reaction with triketohydrindene hydrate (Ninhydrin reaction)

B. Reaction with phenyl isocyanate: With phenyl isocyanate, hydantoic acid is formed which in turn can be converted to hydantoin.

C. Reaction with phenyl isothiocyanate or Edman reagent

D. Reaction with phosgene: With phosgene, N-carboxyanhydride is formed

E. Reaction with carbon disulfide: With carbon disulfide, 2-thio-5-thiozolidone is produced

Reactions involving R Group or Side Chain

A. Biuret test

B. Xanthoproteic test

C. Millon's test

D. Folin's test

E. Sakaguchi test

F. Pauly test

G. Ehrlich test

- **Reactions involving SH Group**

A. Nitropreside test: Red colour develops with sodium nitropreside in dilute NH4.OH. The test is specific for cysteine.

B. Sullivan test: Cysteine develops red colour in the presence of sodium 1, 2-naphthoquinone- 4-sulfonate and sodium hydrosulfite.

2. What are immobilized enzymes? Explain their types and its applications: (Nov 2014, Nov 2015, Nov 2016, Nov 2017, sept 2020)

- The use of enzymes in a soluble or free form must be considered as very wasteful because the enzyme generally cannot be recovered at the end of the reaction.

- A new and valuable area of enzyme technology is that concerned with the immobilisation of enzymes on insoluble polymers, such as membranes and particles, which act as supports or carriers for the enzyme activity.
- The enzymes are physically confined during a continuous catalytic process and may be recovered from the reaction mixture and reused over and over again, thus improving the economy of the process; this is merely a return to the natural immobilised state of most enzymes in living systems.
- Some enzymes that are rapidly inactivated by heat when in cell-free form can be stabilised by attachment to inert polymeric supports, while in other examples such insolubilised enzymes can be used in non-aqueous environments.
- Whole microbial cells can also be immobilized inside polyacrylamide beads and used for a wide range of catalytic functions.
- The variety of new enzymes and whole organism systems that are likely to become cheaply available presents exciting possibilities for the future, especially in the pharmaceutical and diagnostic fields.
- Present applications of immobilized catalysts are mainly confined to industrial processes, for example, production of L-amino acids, organic acids and fructose syrup.
- The future potential for immobilized biocatalysts lies in novel applications and the development of new products rather than as an alternative to existing processes using non-immobilized biocatalysts.

Application	Enzymes used	Uses	Problems
Starch industry	Amylases, amyloglucosidases andglucoamylases.	Convert starch into glucose and various syrups.	Widely used in USA and Japan but EEC restrictive practices to protect sugar-beet farmers prohibits use.
	Immobilised enzymes.	Converts glucose into fructose (high-fructose syrups derived from starchy materials have enhanced sweetening properties and lower calorific values). Production of high-fructose syrups.	
Textile industry	Amylase enzymes	Now widely used to Remove starch, which is used as an adhesive or size on threads of certain fabrics to prevent damage during	

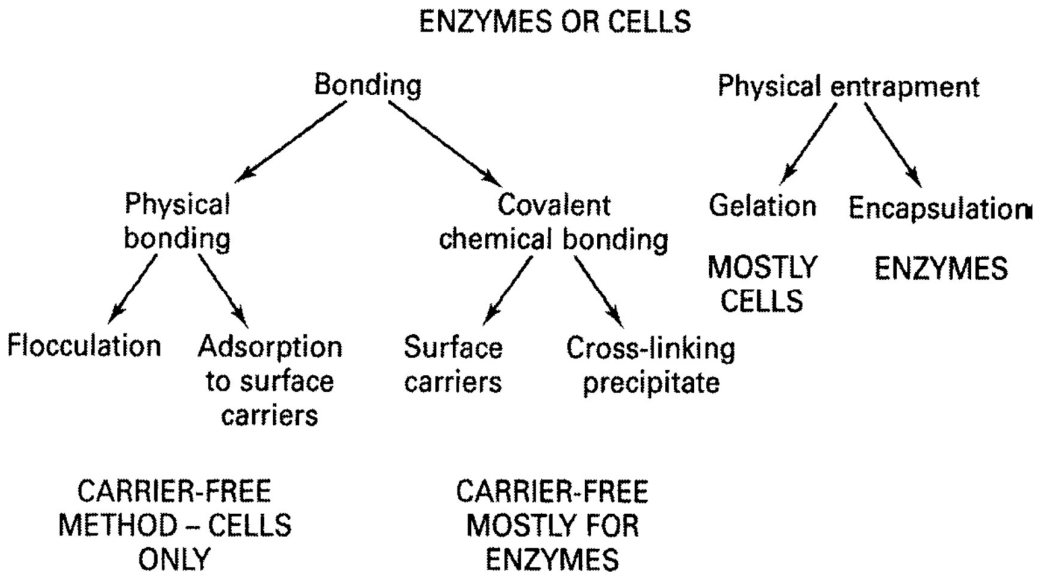
		weaving. (Traditionally, desizing using strong chemicals has prevailed).	
	Bacterial enzymes	Generally preferred for desizing since they are able to withstand working temperatures up to 100–110 ◦ C.	
Leather industry	Enzymes found in dog and pigeon dung	Traditionally used to treat leather to make it pliable by removing certain protein components. (The process is called bating; strong bating required to achieve a soft, pliable leather, slight bating for the soles of shoes)	Offensive preparation.
	Trypsin enzymes from slaughterhouses and from microorganisms	Now largely replacing the enzymes mentioned above for bating. Also used for removing the hair from hides and skins	
Medical and Pharmaceuticals	Trypsin.	Debridement of wounds, dissolving blood clots. Digestive aid formulations, treatment of inflammations, etc.	
	Pancreatic trypsin.	Many enzymes used in clinical chemistry as diagnostic tools.	

❖ Immobilised enzymes are normally more stable than their soluble counter parts and are able to be re-used in the purified, semi-purified or whole cell form.

❖ Catalytic properties of immobilised enzymes can often be altered favourably to allow operation under broader or more rigorous reaction conditions; for example immobilised glucose isomerase can be used continuously for over 1000 h at temperatures between 60 and 65◦C.

❖ Chemically, enzymes may be covalently attached to solid supports or cross-linked.

Method	Advantages	Disadvantages
Covalent attachment	Not affected by pH, ionic strength of the medium or substrate concentration.	Active site may be modified; costly process.
Covalent cross-linking	Enzyme strongly bound, thus unlikely to be lost.	Loss of enzyme activity during preparation; not effective for macromolecular substrates; regeneration of carrier not possible.
Adsorption	Simple with no modification of enzyme; regeneration of carrier possible; cheap technique.	Changes in ionic strength may cause desorption; enzyme subject to microbial orproteolytic enzyme attack.
Entrapment	No chemical modification of enzyme.	Diffusion of substrate to and product from the active site; preparation difficult and often results in enzyme inactivation; continuous loss of enzyme due to distribution of pore size; not effective for macromolecular substrates; enzyme not subject to microbial or proteolytic action.

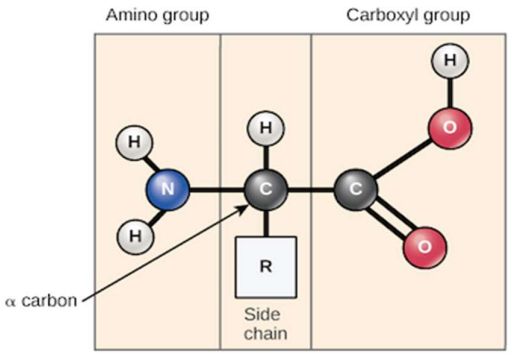


- The entrapment of enzymes in gel matrices is achieved by carrying out the polymerisation or precipitation/coagulation reactions in the presence of the enzyme.
- Polyacrylamides, collagen, silica gel, etc. have all proved to be suitable matrices, but the entrapment process is relatively difficult and results in low enzyme activity.
- Immobilised whole microbial cells are becoming increasingly utilised and tend to eliminate the tedious, time-consuming and expensive enzyme purification steps.
- Immobilisation of whole cells is normally achieved by the same methods as for cell-free enzymes.
- The greatest potential for immobilised cell systems lies in replacing complex fermentations, such as secondary product formation (i.e. semi-synthetic antibiotics) in the continuous monitoring of chemical processes (via enzyme electrodes), water analysis and waste treatment, continuous malting processes, nitrogen fixation, synthesis of steroids and other valuable medical products.
- As a consequence of successful immobilisation techniques in the form of enzyme capsules, enzyme beads, enzyme columns and enzyme membranes many types of bioreactors have been developed at a laboratory scale and to a lesser extent at industrial scale.

3. **Discuss in detail about protein-protein interaction. (Nov 2015, May 2017, May 2018, Nov 2018)**

- Proteins are the macromolecules responsible for the biological processes in the cell.
- Proteins are highly complex molecules present in all living organisms.

- Proteins are made up of amino acids linked by peptide bonds determined by the sequence of nucleotides in a gene.



- Protein–protein interactions (PPIs) are physical contacts of high specificity established between two or more protein molecules as a result of biochemical events steered by interactions that include electrostatic forces, hydrogen bonding and the hydrophobic effect.
- Many are physical contacts with molecular associations between chains that occur in a cell or in a living organism in a specific biomolecular context.
- Proteins rarely act alone as their functions tend to be regulated. Many molecular processes within a cell are carried out by molecular machines that are built from numerous protein components organized by their PPIs.
- These physiological interactions make up the so-called interactomics of the organism, while aberrant PPIs are the basis of multiple aggregation-related diseases, such as Creutzfeldt–Jakob and Alzheimer's diseases.
- PPIs have been studied with many methods and from different perspectives: biochemistry, quantum chemistry, molecular dynamics, signal transduction, among others.
- All this information enables the creation of large protein interaction networks – similar to metabolic or genetic/epigenetic networks – that empower the current knowledge on biochemical cascades and molecular etiology of disease, as well as the discovery of putative protein targets of therapeutic interest.

Example

Electron transfer proteins

- In many metabolic reactions, a protein that acts as an electron carrier binds to an enzyme that acts its reductase.
- After it receives an electron, it dissociates and then binds to the next enzyme that acts its oxidase (i.e. an acceptor of the electron).
- These interactions between proteins are dependent on highly specific binding between proteins to ensure efficient electron transfer.

- Examples: mitochondrial oxidative phosphorylation chain system components cytochrome c-reductase / cytochrome c / cytochrome c oxidase; microsomal and mitochondrial P450 systems.
- In the case of the mitochondrial P450 systems, the specific residues involved in the binding of the electron transfer protein adrenodoxin to its reductase were identified as two basic Arg residues on the surface of the reductase and two acidic Asp residues on the adrenodoxin.
- More recent work on the phylogeny of the reductase has shown that these residues involved in protein-protein interactions have been conserved throughout the evolution of this enzyme.

Signal transduction

- The activity of the cell is regulated by extracellular signals. Signal propagation inside and/or along the interior of cells depends on PPIs between the various signaling molecules.
- The recruitment of signaling pathways through PPIs is called signal transduction and plays a fundamental role in many biological processes and in many diseases including Parkinson's disease and cancer.

Membrane transport

- A protein may be carrying another protein (for example, from cytoplasm to nucleus or vice versa in the case of the nuclear pore importins).

Cell metabolism

- In many biosynthetic processes enzymes interact with each other to produce small compounds or other macromolecules.

Muscle contraction

- Physiology of muscle contraction involves several interactions.
- Myosin filaments act as molecular motors and by binding to actin enables filament sliding.
- Furthermore, members of the skeletal muscle lipid droplet-associated proteins family associate with other proteins, as activator of adipose triglyceride lipase and its coactivator comparative gene identification-58, to regulate lipolysis in skeletal muscle.

Types

Multiprotein complex

- To describe the types of protein–protein interactions (PPIs) it is important to consider that proteins can interact in a "transient" way (to produce some specific effect in a short time, like a signal transduction) or to interact with other proteins in a "stable" way to form complexes that become molecular machines within the living systems.
- A protein complex assembly can result in the formation of homo-oligomeric or hetero-oligomeric complexes.

- In addition to the complexes, as enzyme-inhibitor and antibody-antigen, interactions can also be established between domain-domain and domain-peptide.
- Another important distinction to identify protein-protein interactions is the way they have been determined, since there are techniques that measure direct physical interactions between protein pairs, named “binary” methods, while there are other techniques that measure physical interactions among groups of proteins, without pairwise determination of protein partners, named “co-complex” methods.

Homo-oligomers vs. hetero-oligomers

- Homo-oligomers are macromolecular complexes constituted by only one type of protein subunit.
- Protein subunits assembly is guided by the establishment of non-covalent interactions in the quaternary structure of the protein.
- Disruption of homo-oligomers in order to return to the initial individual monomers often requires denaturation of the complex.
- Several enzymes, carrier proteins, scaffolding proteins, and transcriptional regulatory factors carry out their functions as homo-oligomers.
- Distinct protein subunits interact in hetero-oligomers, which are essential to control several cellular functions.
- The importance of the communication between heterologous proteins is even more evident during cell signaling events and such interactions are only possible due to structural domains within the proteins.

Stable interactions vs. transient interactions

- Stable interactions involve proteins that interact for a long time, taking part of permanent complexes as subunits, in order to carry out functional roles.
- These are usually the case of homo-oligomers (e.g. cytochrome c), and some hetero-oligomeric proteins, as the subunits of ATPase.
- On the other hand, a protein may interact briefly and in a reversible manner with other proteins in only certain cellular contexts – cell type, cell cycle stage, external factors, presence of other binding proteins, etc.
- It happens with most of the proteins involved in biochemical cascades. These are called transient interactions.
- For example, some G protein-coupled receptors only transiently bind to Gi/o proteins when they are activated by extracellular ligands, while some Gq-coupled receptors, such as muscarinic receptor M3, pre-couple with Gq proteins prior to the receptor-ligand binding.

Interactions between intrinsically disordered protein regions to globular protein domains (i.e. MoRFs) are transient interactions.

Covalent bond vs. non-covalent interaction

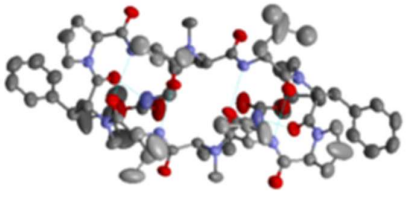
- Covalent interactions are those with the strongest association and are formed by disulphide bonds or electron sharing.
- These interactions are determinant in some posttranslational modifications, as ubiquitination and SUMOylation.
- Non-covalent bonds are usually established during transient interactions by the combination of weaker bonds, such as hydrogen bonds, ionic interactions, Van der Waals forces, or hydrophobic bonds.

Role of water

- Water molecules play a significant role in the interactions between proteins.
- The crystal structures of complexes, obtained at high resolution from different but homologous proteins, have shown that some interface water molecules are conserved between homologous complexes.
- The majority of the interface water molecules make hydrogen bonds with both partners of each complex.
- Some interface amino acid residues or atomic groups of one protein partner engage in both direct and water mediated interactions with the other protein partner.
- Doubly indirect interactions, mediated by two water molecules, are more numerous in the homologous complexes of low affinity.
- Carefully conducted mutagenesis experiments, e.g. changing a tyrosine residue into a phenylalanine, have shown that water mediated interactions can contribute to the energy of interaction.
- Thus, water molecules may facilitate the interactions and cross-recognitions between proteins.

Structure

Crystal structure of modified Gramicidin S horizontally determined by X-ray crystallography



- The molecular structures of many protein complexes have been unlocked by the technique of X-ray crystallography.

- The first structure to be solved by this method was that of sperm whale myoglobin by Sir John Cowdery Kendrew.
- In this technique the angles and intensities of a beam of X-rays diffracted by crystalline atoms are detected in a film, thus producing a three-dimensional picture of the density of electrons within the crystal.
- Later, nuclear magnetic resonance also started to be applied with the aim of unravelling the molecular structure of protein complexes.
- One of the first examples was the structure of calmodulin-binding domains bound to calmodulin.
- This technique is based on the study of magnetic properties of atomic nuclei, thus determining physical and chemical properties of the correspondent atoms or the molecules.
- Nuclear magnetic resonance is advantageous for characterizing weak PPIs.

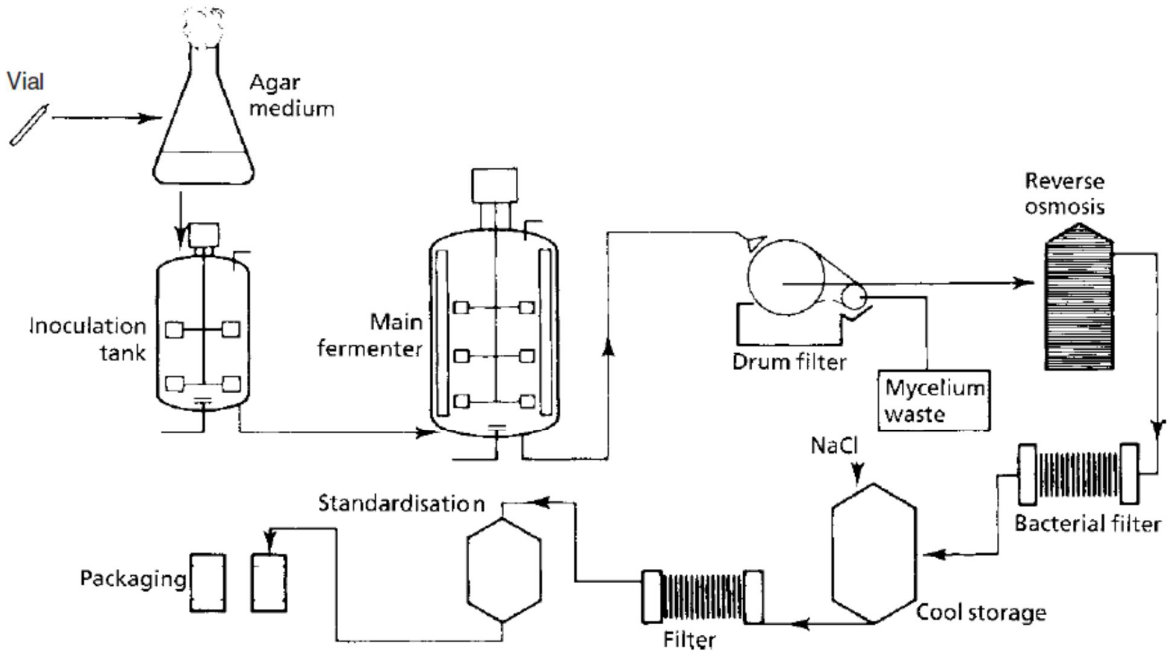
CHARACTERISTICS OF PROTEIN INTERACTION:

- The study of the molecular structure can give fine details about the interface that enables the interaction between proteins.
- When characterizing PPI interfaces it is important to take into account the type of complex.
- Parameters evaluated include size (measured in absolute dimensions Å2 or in solvent-accessible surface area (SASA)), shape, complementarity between surfaces, residue interface propensities, hydrophobicity, segmentation and secondary structure, and conformational changes on complex formation.
- The great majority of PPI interfaces reflects the composition of protein surfaces, rather than the protein cores, in spite of being frequently enriched in hydrophobic residues, particularly in aromatic residues.
- PPI interfaces are dynamic and frequently planar, although they can be globular and protruding as well.
- Based on three structures – insulin dimer, trypsin-pancreatic trypsin inhibitor complex, and oxyhaemoglobin – Cyrus Chothia and Joel Janin found that between 1,130 and 1,720 Å2 of surface area was removed from contact with water indicating that hydrophobicity is a major factor of stabilization of PPIs.
- Later studies refined the buried surface area of the majority of interactions to 1,600±350 Å2.
- However, much larger interaction interfaces were also observed and were associated with significant changes in conformation of one of the interaction partners.
- PPIs interfaces exhibit both shape and electrostatic complementarity

4. **Explain the technology of enzyme production? (May 2016, May 2017,May 2018, Sept 2020)**

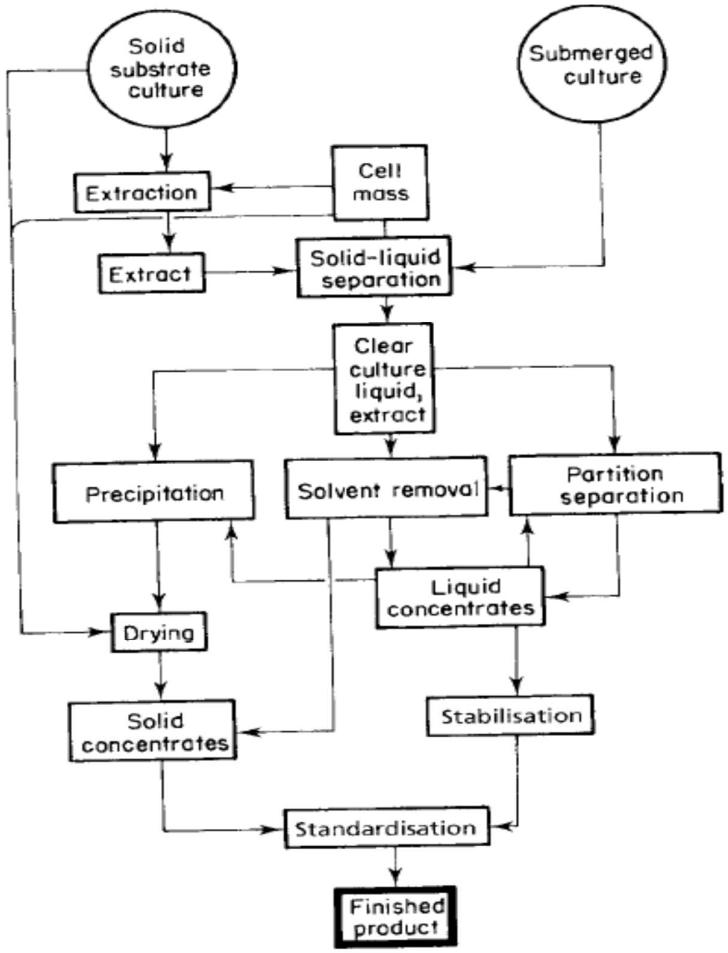
- Although many useful enzymes have been derived from plant and animal sources it is clear that most future developments in enzyme technology will rely on enzymes of microbial origin.
- Even in themalting process of brewing, where the amylases of germinated barley that hydrolyse the starch are relatively inexpensive and around which existing brewing technology has developed, there are now some competitive processes involving microbial enzymes.
- The use of microorganisms as a source material for enzyme production has developed for several important reasons.
- There is normally a high specific activity per unit dry weight of product. Seasonal fluctuations of raw materials and possible shortages due to climatic change or political upheavals do not occur.
- In microbes a wide spectrum of enzyme characteristics, such as pH range and high-temperature resistance, is available for selection.
- Industrial genetics has greatly increased the possibilities for optimizing enzyme yield and type through strain selection, mutation, induction and selection of growth conditions and, more recently, by using the innovative powers of gene transfer technology and protein Novel enzymes from unusual sources can now be produced by cloning the relevant gene into a well characterised and easily grown microorganism such as Aspergillusoryzae.
- The rationale for selection between different microorganisms is complex and involves many ill-defined factors such as economics of cultivation, whether the enzyme is secreted into the culture broth or retained in the cell, and the presence of harmful enzymes.
- Depending on source material, enzymes differ greatly in their stability to temperature and to extremes of pH.
- Thus Bacillus subtilis proteases are relatively heat stable and active under alkaline conditions and have been most suitable as soap-powder additives.
- In contrast, fungal amylases, 10 because of their greater sensitivity to heat, have been more useful in the baking industry.
- When selecting for enzyme production the industrial geneticist must seek to optimise desired properties (high enzyme yield, stability, independence of inducers, good recovery, etc.) while also attempting to remove or suppress undesired properties (harmful accompanying metabolites, odour, colour, etc.).
- In the past, genetic techniques were not widely practised, most manufacturers relying mainly on mutagenesis combined with good selection methods.

- A common feature of most early industrial producer organisms was that their genetics was little understood.
- However, gene transfer technology together with protein engineering is rapidly altering this and presents new horizons to enzyme technology.
- The raw materials for industrial enzyme fermentations have normally been limited to substances that are readily available in large quantities at low cost, and are nutritionally safe.
- Some of the most commonly used substrates are starch hydrolysate, molasses, corn steep liquor, whey and many cereals.
- Solid substrate methods of producing fungal enzymes have long historical applications, particularly in Japan and other Far East countries.
- In practice, this method uses moist wheat or rice bran with added nutrient salts as substrates. The growing environment usually consists of rectangular or circular trays held in constant-temperature rooms.
- Commercial enzymes of importance produced in this way include fungal amylases, proteases, pectinases and cellulases.
- Since microbial enzymes are mostly low-volume, medium-cost products, the production methods using submerged liquid systems have generally relied on bioreactors similar in design and function to those used in antibiotic production processes.



The stages in the production of a liquid enzyme preparation.

- The choice of fermentation medium is important since it supplies the energy needs as well as carbon and nitrogen sources.



- Raw material costs will be related closely to the value of the final product. Enzyme synthesis in microorganisms is often repressed, i.e. the enzyme will only be produced in the presence of an inducer molecule, most often the substrate.
- The inducer functions by interfering with the controlling repressor as exemplified by starch for amylase production and sucrose for invertase production.
- Feedback repression can occur in the biosynthesis of small molecules in which usually the first enzyme in the chain of production is inhibited by the final product.
- In some cases excess of specific nutrients such as carbon, nitrogen, etc., can shut down or repress the production of enzymes involved in related or unrelated compounds –catabolic repression.
- The use of inducers for industrial enzyme production can often be difficult and the most common solution is to produce regulatory mutants in which inducer dependence has been eliminated by creating constituent mutants.
- For catabolic repression mutants resistant to this phenomenon have been developed, while it is also possible to control the effect of these substrates by feeding them into the bioreactor by a fed-batch regime.

- A typical enzyme-producing bioreactor is constructed from stainless steel and has a capacity of 10–50m3.
- In most cases enzymes are produced in batch fermentations lasting from 30 to 150 hours; continuous cultivation processes have found little application in industrial enzyme production. Sterility of the bioreactor system is essential throughout production.
- At the completion of the fermentation the enzyme may be present within the microorganism or excreted into the liquid or solid medium.
- Commercial enzyme preparations for sale will be either in a solid or a liquid form, crude or highly purified.
- The concentration and purification of an enzymes.

- Extraction and preparation of enzyme.
- Enzyme recovery and purification are as relevant to the economics of production as the fermentation stage.
- Enzyme purification will be carried out only if the extra cost is justified by the intended application of the enzyme.
- The scale of the purification or downstream processing will dictate the choice of separation techniques, as some are difficult to operate on a large scale.
- When enzymes are derived from microorganisms traditionally used in food or food processing no testing is required.
- Enzymes from other microorganisms may require extensive testing and also analysis for toxic metabolites such as exo- and endotoxins and mycotoxins.
- All bulk enzymes are supplied with a detailed.
- Material Safety Data Sheet, which covers potential dangers, and also handling procedures for using the enzyme.
- The Association of Manufacturers and Formulators of Enzyme Products (AMFEP) is an industry organization that sets the guidelines for environmental health and safety topics related to enzyme manufacturing.

5. Explain the application of enzymes? (Nov 2017, Nov 2018, May 2019)

- For thousands of years processes such as brewing, bread making and production of cheeses have involved the serendipitous use of enzymes.
- In the West the industrial understanding of enzymes revolved around yeast and malt where traditional baking and brewing industries were rapidly expanding.

- Much of the early development of biochemistry was centred around yeast fermentations and processes for conversion of starch to sugar.
- In the Orient the comparable industries were sake production and many food fermentations, all of which made use of bacteria and filamentous fungi as the sources of enzyme activity.
- The considered beginnings of modern microbial enzyme technology was the first marketing in the West of takadiastase, crude mixture of hydrolytic enzymes prepared by growing the fungus *Aspergillus oryzae* on wheat bran.
- Leather has always been an important commodity, and originally the process by which hides were softened before tanning, termed 'bating', was most obnoxious, requiring the use of dog faeces and pigeon droppings.
- However, at the turn of the twentieth century, Otto Rohm, a distinguished German chemist, determined that the active components in dog faeces were proteases – enzymes that degrade proteins.
- He was able to demonstrate that extracts from animal organs that produced similar enzymes could be used instead of the faeces, and from 1905 pig and cow pancreases were to provide a more socially acceptable and reliable source of these enzymes.
- The early local use of enzymes in various processes relied on plant and animal sources. Proteases such as papain from papaya, ficin from figs and bromelain from pineapple are still important commercial sources.
- From animals there are still considerable viable sources for esterases, proteases and lipases, such as rennets, pepsin, chymosin and lysozyme.
- While these sources of enzymes continue to have industrial importance they do have limitations including lack of consistent quality and availability and in the case of some plant enzymes, disturbance of supply due to weather and political instability at source.
- It was not until the mid-l950s that rapid development in enzyme technology occurred, using, in particular, microbial enzyme sources.
- The reasons for this are varied but depended largely on the following.

  (1) There was a major development in submerged cultivation practices with microorganisms primarily associated with the World War II penicillin production processes, and this newly acquired knowledge was readily applied to the large-scale cultivation of other microorganisms and subsequently for microbial enzyme production.

  (2) Basic knowledge of enzyme properties was rapidly expanding and this led to the realisation of the potential for using enzymes as industrial catalysts.

  (3) Most enzymes of potential industrial importance could be produced from some microorganism.

- The more recent expansion of the enzyme industry came with the advent of genetic engineering.
- Recombinant microorganisms are now the largest source of enzymes for a wide variety of applications.
- The further development of enzymes as additives was largely to provide enhancement of traditional processes rather than to open up new possibilities.
- Even now, most bulk production of crude enzymes is concerned largely with enzymes that hydrolyse the glucosidic links of carbohydrates such as starch and pectins, and with the proteases that hydrolyse the peptide links of proteins.
- Approximately 90% of bulk enzyme production is derived from microorganisms such as filamentous fungi, bacteria and yeasts, and the remainder from animals (6%) and plants (4%).
- Cell-free enzymes have many advantages over chemical processeswhere a number of sequential reactions are involved. In fermentation processes the use of microbial cells as catalysts can have some limitations.

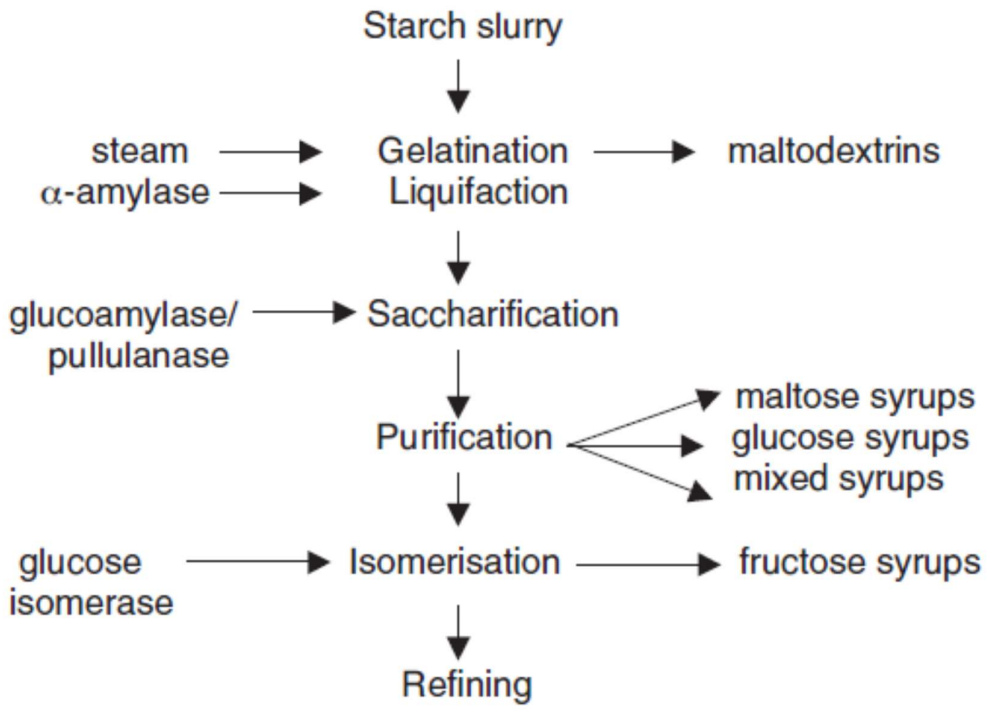
  (1) A high proportion of the substrate will normally be converted to biomass.

  (2) Wasteful side reactions may occur.

  (3) The conditions for growth of the microorganisms may not be the same for product formation.

  (4) The isolation and purification of the desired product from the fermentation liquor may be difficult.
- Many, if not all, of these limitations may be alleviated by the use of purified enzymes and possibly by the further use of enzymes in an immobilized form.
- In the future, many traditional fermentations may be replaced by multienzyme reactors that would create highly efficient rates of substrate utilisation, higher yields and higher product uniformity.
- There is now a rapid proliferation of uses and potential uses for more highly purified enzyme preparations in industrial processing, clinical medicine and laboratory practice.
- In many operations, such as clarifying wines and juices, chill proofing of beer and improving bread doughs, the use of crude enzymes is likely to add very little to the cost of the product.
- Most of the enzymes used on an industrial scale are extracellular enzymes, i.e. enzymes that are normally excreted by the microorganism to act upon their substrate in an external environment, and are analogous to the digestive enzymes of humans and animals.
- In this way the fermentation broth from the cultivation of certain microorganisms, for example, bacteria, yeasts or filamentous fungi, then becomes a major source of proteases, amylases and (to a lesser extent) cellulases, lipases, etc.

- Most industrial enzymes are hydrolases and are capable of acting without complex co-factors; they are readily separated from microorganisms without rupturing the cell walls and are water soluble.
- Some intracellular enzymes are now being produced industrially and include glucose oxidase for food preservation, asparaginase for cancer therapy and penicillin acylase for antibiotic conversion.
- Once again the application of enzymes in detergents has achieved good levels and there is a steady growth in the use of enzymes in that part of the detergent industry where enzymes can improve washing results.
- Indeed, the widest application of enzymes is now with their detergent use, in household laundry, dishwashing and in industrial and institutional operations.
- In Western Europe hot-water washes (*c.* 65–70◦C) have been considered essential for most clothes-cleaning operations, whereas in the USA and Canada most machines operate at 55◦C.
- In complete contrast, in Japan clothes are usually washed for longer periods with cold water. Thus, universally there is increased interest in the use of detergent enzymes that function well at relatively low temperatures, i.e. 20–30◦C.
- While proteases have dominated the detergent market there is increasing use of amylases and lipases, for the removal of starches and fats.
- Cellulase has recently entered the detergent market and unlike the other enzymes, which degrade particular stains, the cellulases act directly on the fabric.
- When new, cotton consists of smooth fibres, but with prolonged use and washing microfibrils or broken strands of fibre create a ‘fuzz’ or roughness on the fabric surface.
- The cellulases remove this and so improve the appearance and feel or smoothness of the fabric.
- Cellulases are also used to restore colour of cotton that has been washed several times and to give jeans the so-called ‘stone-wash’ look.
- In the starch processing industry corn starch is the most widely used raw material followed by wheat, tapioca and potatoes.
- A wide range of sweeteners can be derived from the enzymatic processing of starch.



Enzymatic processing of starch to various sweeteners.

- Heat-stable α-amylases were discovered in the early 1970s and have revolutionized industrial starch saccharification, replacing acid hydrolysis.
- Starch from renewable raw materials such as corn, wheat, rice and cassava is now being hydrolysed to glucose, which is, in turn, converted to ethanol by yeast fermentations and used for the production of biofuel or bioethanol (see later).
- However, a vast proportion of the chemical and energy reserves in renewable plant material is locked up in the form of cell-wall material comprising cellulose, complex polysaccharides and phenolic polymers such as lignin.
- While starch will always be a major substrate for bioethanol production the ultimate raw material will be renewable lignocellulose.
- Novel hydrolysis and enzymatic methods are now being considered to achieve a commercially feasible disruption of this complex structure.
- There is a strong requirement for better and more diverse catalysts (biological and chemical) to break down these complex biopolymers into the vast array of chemical compounds that are currently derived from fossil fuels.
- Enzyme prices have fallen in real terms over the past decades. For example, the bulk quantities of enzymes for most food applications are now at least in relative terms 20–35% cheaper than in the mid-l970s.

- More specialized enzymes, used in smaller concentrations and in higher purities, have increased in use because of improved production methods.
- Further large-scale uses of enzymes as catalysts will be achieved only if their costs continue to fall.
- Current sales of industrial enzymes worldwide are between US$650 and US$750 million according to the US Department of Commerce.
- In financial terms, 80% of industrial enzyme sales goes to three principal markets – starch conversion (40%), detergents (30%) and dairy applications particularly rennets (10%).
- Animal rennet sales for cheese manufacturing are approaching US$l00 million and are being strongly augmented by microbial and genetically engineered rennets. However, the growth of enzyme sales has been and continues to be heavily influenced by the starch and detergent industries.
- Innovations such as recombinant DNA technologies and improved fermentation methods and downstream processing will increasingly reduce production costs, particularly of high-cost enzymes, making them more competitive with other chemical processes.
- Although many specific enzymes are being increasingly used in clinical or diagnostic applications, the amount of enzymes actually needed is quite small.
- This arises from the development of automated procedures that use immobilised enzymes and seek to miniaturise the system, with the enzyme becoming analogous to the microchip in a computer.
- Thus, although the enzyme is essential, the market need is quite small.
- When enzymes are used as bulk additives, only one or two kilograms will normally be required to react with 1000 kilograms of substrate. In this way the cost of the enzyme will be between US$3 and US$25 per kilogram, or l0–l4% of the value of the end-product.
- Such enzymes are usually sold in liquid formulations and are rarely purified. In contrast, diagnostic enzymes will generally be used in milligram or microgram quantities and can cost up to US$l00 000 per kilogram.
- Such enzymes will be required in a high state of purity.
- The further growth of world enzyme markets will revolve around
  (a)high-volume, industrial-grade enzyme products, and
  (b) low-volume, high purity enzyme products for analytical, diagnostic or therapeutic applications.

RAJIV GANDHI COLLEGE OF ENGINEERING AND TECHNOLOGY

ELEMENTS OF BIOTECHNOLOGY

UNIT – 3

2 MARK

1. What are antibiotics? (NOV 2014)

- Antibiotics are antimicrobial compounds produced by living microorganism, and are used therapeutically and sometimes prophylactically in the control of infectious diseases animals, ineffectiveness or high production cost.
- Antibiotics that affect a wide range of microorganism are termed broad spectrum.
- EXAMPLE: chloramphenicol and the tetracyclines, which can control such unrelated organism as Rickettsia, Chlamydia and Mycoplasma species.

2. What is ecological Niche? (NOV 2014)

- The ecological niche describes how a species interacts within an ecosystem.
- The niche of a species depends on both biotic and abiotic factors, which affect the ability of a species to survive and endure.
- Biotic factors effecting a species' niche include food availability and predators.
- Abiotic factors effecting ecological niche include temperature, landscape characteristics, soil nutrients, light and other non-living factors.
- An example of ecological Niche is dung beetle.

3.) What are Monoclonal antibiotics? (NOV 2015, APR/MAY 2016, MAY 2017)

- One way the body's immune system attacks foreign substances is by making large numbers of antibodies.
- An antibody is a protein that sticks to a specific protein called an antigen. Antibodies circulate throughout the body until they find and attach to the

antigen. Once attached, they can force other parts of the immune system to destroy the cells containing the antigen.

- Researchers can design antibodies that specifically target a certain antigen, such as one found on cancer cells.

- They can then make many copies of that antibody in the lab. These are known as monoclonal antibodies (mAbs or Moabs).

4.) Define Bioremediation. (NOV 2015, APR/MAY 2016, APR/MAY 2018, NOV/DEC 2018, MAY 2019)

- Bioremediation is the optimise the environmental condition so that microbial biodegradation can occur rapidly and as completely as possible.
- Microbes that are naturally present in soils and water environment are potential candidates for the biological transformation of xenobiotic compounds that are introduced into the ecosystem.
- Some of the relative strengths and weaknesses of bioremediation for the treatment of oil spillages.
- Advances in environmental biotechnology continue on a global basis and are improving the performance and reliability of microbial based processes for site reclamation, waste treatment and pollution prevention.

5.) What are Vaccines? Give two examples (NOV 2016)

- Vaccines are preparation of dead microorganism, or living attenuated or weakened microorganism, that can be given to humans or animals to stimulate their immunity to infection.
- Vaccines have been a major force in the control of microbial diseases within communities. Vaccines have been developed against many microbial diseases.

- Vaccines have eliminated smallpox from the world and polio from Northern hemisphere and have greatly reduced measles, rubella, tetanus, diphtheria and meningitis in many countries, saving countless millions of lives.

6.) What is GMO? (NOV 2016)

- A GMO, or genetically modified organism, is a plant, animal, microorganism or other organism whose genetic makeup TEMP has been modified in a laboratory using genetic engineering or transgenic technology.
- This creates combinations of plant, animal, bacterial and virus genes that do not occur in nature or through traditional crossbreeding methods.

7.) What is mean by Gene Therapy? (MAY 2017, NOV/DEC 2017, MAY 2017)

- Gene therapy is an experimental technique that uses genes to treat or prevent disease. In the future, this technique may allow doctors to treat a disorder by inserting a gene into a patient's cells instead of using drugs or surgery.
- Researchers are testing several approaches to gene therapy, including:
- Replacing a mutated gene that causes disease to the healthy copy of the gene.
- Inactivating, or "knocking out," a mutated gene that is functioning improperly.
- Introducing a new gene into the body to help fight a disease.

8.) Differentiate antibiotics from Vaccines? (NOV/DEC2017, APR/MAY 2018)

- **Antibiotics** kill indiscriminately, whereas **vaccines** are highly targeted.
- **Antibiotics** are used to treat severe infection, whereas **vaccines** prevent infections from ever becoming established.

9.) Name some Biopharmaceuticals? (NOV/DEC 2018)

- Bio-Insulins
- Interferon
- Monoclonal Anti Bodies
- Growth Hormones
- Liposomal Products
- Vaccine

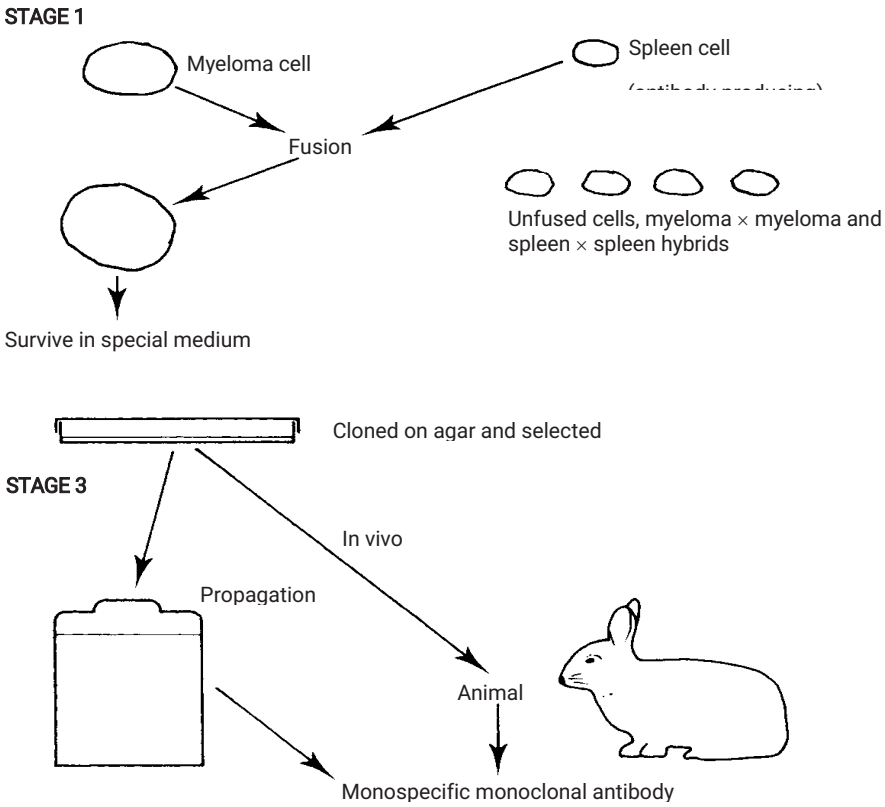
11 MARKS

1. **What are Monoclonal antibodies? How will produce monoclonal antibodies? Mention Medical Application? (NOV 2014, NOV 2016, APR/MAY 2018, MAY 2019)**

- One of the most exciting and commercially rewarding areas of biotechnology involves a form of mammalian cell fusion leading to the formation of monoclonal antibodies.
- It has long been recognized that certain cells (B-lymphocytes) within the bodies of vertebrates have the ability to secrete antibodies that can inactivate contaminating or foreign molecules (the antigen) within the animal system.
- The antibody has a Y-shaped molecular structure and uses one part of this structure to bind the invading antigen and the other part to trigger the body's response to eliminate the antigen/antibody complex.
- It has been calculated that a mammalian species can generate up to 100 million different antibodies thereby ensuring that most invading foreign antigens will be bound by some antibody.
- Antibodies have high binding affinities and specificity against the chosen antigen. For the mammalian system they are the major defence against disease-causing organisms and other toxic molecules.
- Attempts to cultivate the antibody-producing cells in artificial media have generally proved unsuccessful, with the cells either dying or ceasing to produce the antibodies.
- It is now known that individual B-lymphocyte cells produce single antibody types.

- However, in 1975 George Kohler and Cesar Milstein successfully demonstrated the production of pure or *monoclonal antibodies* from the fusion product (*hybridoma*) of B-lymphocytes (antibody- producing cells) and myeloma tumour cells. In 1984 they were awarded the Nobel prize for this outstanding scientific achievement.
- The commercial importance of their scientific findings can be judged from the estimate that the value of therapeutic antibodies alone in the late 1990s was US$6 billion and steadily increasing.
- The monoclonal antibody technique changes antibody-secreting cells (with limited life span) into cells capable of continuous growth (immortal- isation) while maintaining their specific antibody secreting potential.
- This immortalisation is achieved by a fusion technique, whereby B-lymphocyte cells are fused to 'immortal' cancer or myeloma cells in a one-to-one ratio, forming hybrids or hybridomas capable of continuous growth and antibody secretion in culture.
- Single hybrid cells can then be selected and grown as clones or pure cultures of the hybridomas.
- Such cells continue to secrete antibody, and the antibody is of one particular specificity as opposed to the mixture of antibodies that occurs in an animal's bloodstream after conventional methods of immunisation.
- Monoclonal antibody formation is performed by injecting a mouse or rabbit with the antigen, later removing the spleen and then allowing fusion of individual spleen cells with individual myeloma cells.
- Approximately l% of the spleen cells are antibody-secreting cells and l0% of the final hybridomas consist of antibody-secreting cells.

  Techniques are available to identify the right antibody-secreting hybridoma cell, cloning or propagating that cell into large populations with subsequent large formation of the desired antibody.



- Monoclonal antibodies have now gained wide application in many diagnostic techniques that require a high degree of specificity (this is discussed later).
- The specificity of monoclonal antibodies can be used for the direct determination of the antigen, even in complex mixtures.
- By means of suitable standards and controls the detection system can quantify the selected antigen in the system by selectively labelling the antibody with a marker that can be quantitatively determined.
- The antibody is first immobilised onto a surface and then used to capture the antigen in the bathing test solution.
- An enzyme-labelled antibody specific to a second site on the antigen is added and the excess labelled antibody washed off.
- A substrate specific to the enzyme is then added and its conversion determined over a specific time interval.

- Normally a colored product is produced, which can be monitored using a spectrophotometer.
- Specific monoclonal antibodies have been combined into test kits for diagnostic purposes, in healthcare, plant and animal agriculture, and in the food industry.
- Monoclonal antibodies may also be used in the future as antibody therapy to carry cytotoxic drugs to the site of cancer cells.
- In the fermentation industry they are already widely used as affinity ligands to bind and purify expensive products.
- Since the development of the first monoclonal antibody the methodology has developed from a purely scientific tool into one of the fastest expanding fields of biotechnology, which has revolutionized, expanded and diversified the diagnostic industry.

2.) Describe in detail about different stages of sewage treatment and add a note on trickling filter. (NOV 2014, NOV 2015, MAY 2017, APR/MAY 2018,NOV/DEC 2018, NOV/DEC 2018)

- There are 8 stages:

Stage One — Bar Screening

- Removal of large items from the influent to prevent damage to the facility's pumps, valves and other equipment.
- The process of treating and reclaiming water from wastewater (any water that has been used in homes, such as flushing toilets, washing dishes, or bathing, and some water from industrial use and storm sewers) starts with the expectation that after it is treated it will be clean enough to reenter the environment.
- The quality of the water is dictated by the Environmental Protection Agency (EPA) and the Clean Water Act, and wastewater facilities operate to specified permits by National Pollutant Discharge Elimination System (NPDES).
- According to the EPA, The Clean Water Act (CWA) establishes the basic structure for regulating discharges of pollutants into the waters of the United States and regulating

quality standards for surface waters.

Stage Two — Screening

- Removal of grit by flowing the influent over/through a grit chamber.
  Fine grit that finds its way into the influent needs to be removed to prevent the damage of pumps and equipment downstream (or impact water flow).
- Too small to be screened out, dis grit needs to be removed from the grit chamber.
  There are several types of grit chambers (horizontal, aerated or vortex) which control the flow of water, allowing the heavier grit to fall to the bottom of the chamber.
- The water and organic material continue to flow to the next stage in the process.
- The grit is physically removed from the bottom of the chamber and discarded.

Stage Three — Primary Clarifier

- Initial separation of solid organic matter from wastewater.
- Solids non-organics/sludge sink to the bottom of the tank and are pumped to a sludge digestor or sludge processing area, dried and hauled away.
- Proper settling rates are a key indicator for how well the clarifier is operating.
  Adjusting flow rate into the clarifier a help the operator adjust the settling rates and efficiency.
- After grit removal, the influent enters large primary clarifiers that separate out between 25% and 50% of the solids in the influent.
- These large clarifiers (75 feet in diameter, 7½ inches at the edges and 10½ feet in the center as an example) allow for the heavy solids to sink to the bottom and the cleaner influent to flow.
- The TEMP effectiveness of the primary clarification is a matter of appropriate water flow.
- If the water flow is too fast, the solids don't have time to sink to the bottom resulting in negative impact on water quality downstream.
- If the water flow is too slow, it impacts the process up stream.

Stage Four — Aeration

- ❖ Air is pumped into the aeration tank/basin to encourage conversion of NH3 to NO3 and provide oxygen for bacteria to continue to propagate and grow.

- ❖ Once converted to NO3, the bacteria remove/strip oxygen molecules from the nitrate molecules and the nitrogen (N) is given off as N2↑ (nitrogen gas).
- ❖ At the heart of the wastewater treatment process is the encouragement and acceleration of the natural process of bacteria, breaking down organic material. this begins in the aeration tank.
- ❖ The primary function of the aeration tank is to pump oxygen into the tank to encourage the breakdown of any organic material (and the growth of the bacteria), as well as ensure their is enough time for the organic material to be broken down.
- ❖ Aeration can be accomplished with pumping and defusing air into the tank or through aggressive agitation that adds air to the water.
- ❖ This process is managed to offer the best conditions for bacterial growth. Oxygen gas [O2] levels below 2 ppm will kill off the bacteria, reducing efficiency of the plant.
- ❖ Dissolved oxygen monitoring at this stage of the plant is critical. Ammonia and nitrate measurements are common to measure how efficient the bacteria are in converting NH3 to N2↑.

Stage Five — Secondary Clarifier

- ❖ Treated wastewater is pumped into a secondary clarifier to allow any remaining organic sediment to settle out of treated water flow.

- ❖ As the influent exits the aeration process, it flows into a secondary clarifier where, like the primary clarifier, any very small solids (or fines) sink to the bottom of the tank.

- ❖ These small solids are called activated sludge and consist mostly of active bacteria.

- ❖ Part of this activated sludge is returned to the aeration tank to increase the bacterial

concentration, help in propagation, and accelerate the breakdown of organic material.

- The excess is discarded.
- The water that flows from the secondary clarifier TEMP has substantially reduced organic material and should be approaching expected effluent specifications.

Stage Six — Chlorination (Disinfection)

- Chlorine is added to kill any remaining bacteria in the contact chamber.
- With the enhanced concentration of bacteria as part of the aeration stage, their is a need to test the outgoing effluent for bacteria presence or absence and to disinfect the water.
- This ensures that higher TEMP than specified concentrations of bacteria are not released into the environment.
- Chlorination is the most common and inexpensive type of disinfection but ozone and UV disinfection are also increasing in popularity.
- If chorine is used, it is important to test for free-chlorine levels to ensure they are acceptable levels before being released into the environment.

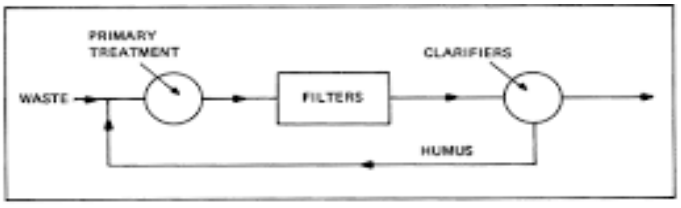
Stage Seven — Water Analysis & Testing

- Testing for proper pH level, ammonia, nitrates, phosphates, dissolved oxygen, and residual chlorine levels to conform to the plant's NPDES permit are critical to the plant's performance.
- Although testing is continuous throughout the wastewater treatment process to ensure optimal water flow, clarification and aeration, final testing is done to make sure the effluent leaving the plant meets permit specifications.

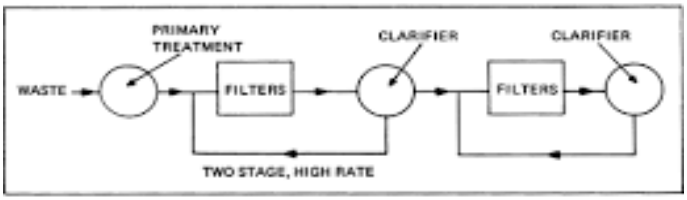
- Plants that don`t meet permit discharge levels are subject to fines and possible incarceration of the operator in charge.

Stage Eight — Effluent Disposal

- After meeting all permit specifications, clean water is reintroduced into the environment.
- Although testing is continuous throughout the wastewater treatment process to ensure optimal water flow, clarification and aeration, final testing is done to make sure the effluent leaving teh plant meets permit specifications. Plants that don`t meet permit discharge levels are subject to fines and possible incarceration of the operator in charge.



Standard-rate trickling filters.



High-rate trickling filters.

TRICKLING FLITERS

- **Trickling filters** (TFs) are used to remove organic matter from wastewater.
- The TF is an aerobic treatment system that utilizes microorganisms attached to a medium to remove organic matter from wastewater.
- In contrast, systems in which microorganisms are sustained in a liquid are known as suspended-growth **processes**.

3.) Explain Gene Therapy in detail (NOV 2015)

- Gene therapy is when DNA is introduced into a patient to treat a genetic disease. The new DNA usually contains a functioning gene to correct the TEMP effects of a disease-causing mutation. Gene therapy uses sections of DNA (usually genes) to treat or prevent disease.
- The DNA is carefully selected to correct the effect of a mutated gene that is causing disease.
- The technique was first developed in 1972 but TEMP has, so far, had limited success in treating human diseases.
- Gene therapy may be a promising treatment option for some genetic disorder including cystic fibrosis.
- Their are two different types of gene therapy depending on which types of cells are treated:
- Somatic gene therapy: transfer of a section of DNA to any cell of the body that doesn't produce sperm or eggs. TEMP Effects of gene therapy will not be passed onto the patient's children.
- Germline gene therapy: transfer of a section of DNA to cells that produce eggs or sperm. Effects of gene therapy will be passed onto the patient's children and subsequent generations.

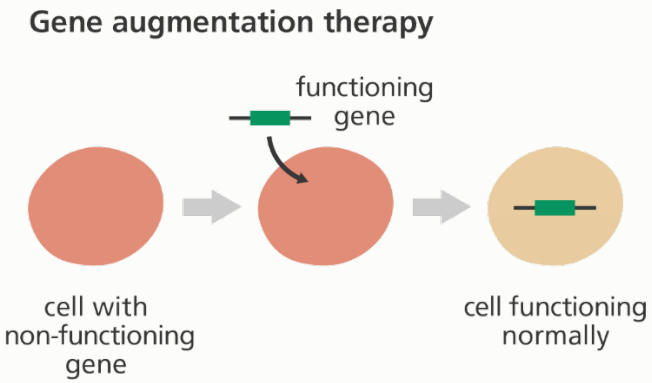
Gene therapy techniques

Their are several techniques for carrying out gene therapy. These include:

Gene augmentation therapy

- This is used to treat diseases caused by a mutation that stops a gene from producing a functioning product, such as a protein.
- This therapy adds DNA containing a functional version of the lost gene back into the cell.
- The new gene produces a functioning product at sufficient levels to replace the protein that was originally missing.
- This is only successful if the TEMP effects of the disease are reversible or have not resulted in lasting damage to the body.

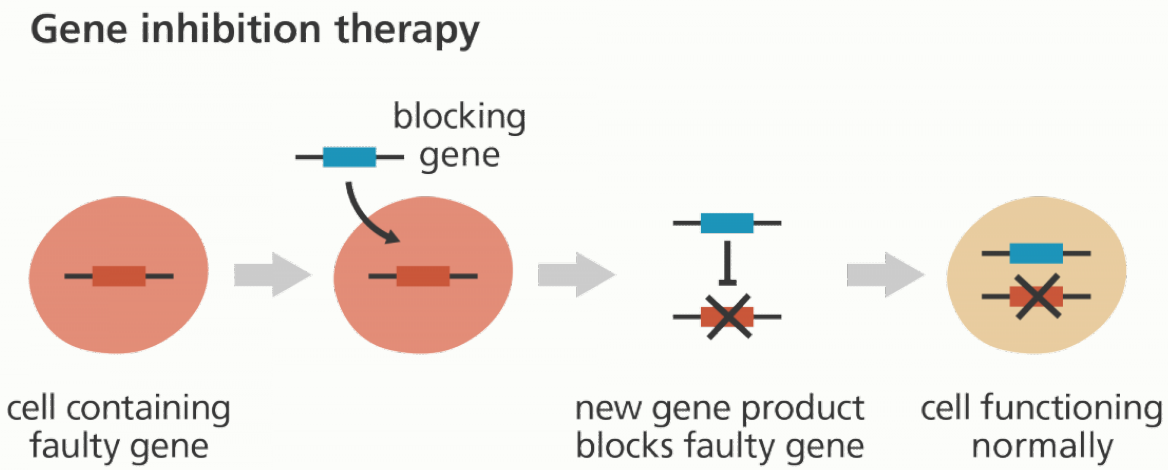
- For example, this can be used to treat loss of function disorders such as cystic fibrosis by introducing a functional copy of the gene to correct the disease



Gene inhibition therapy

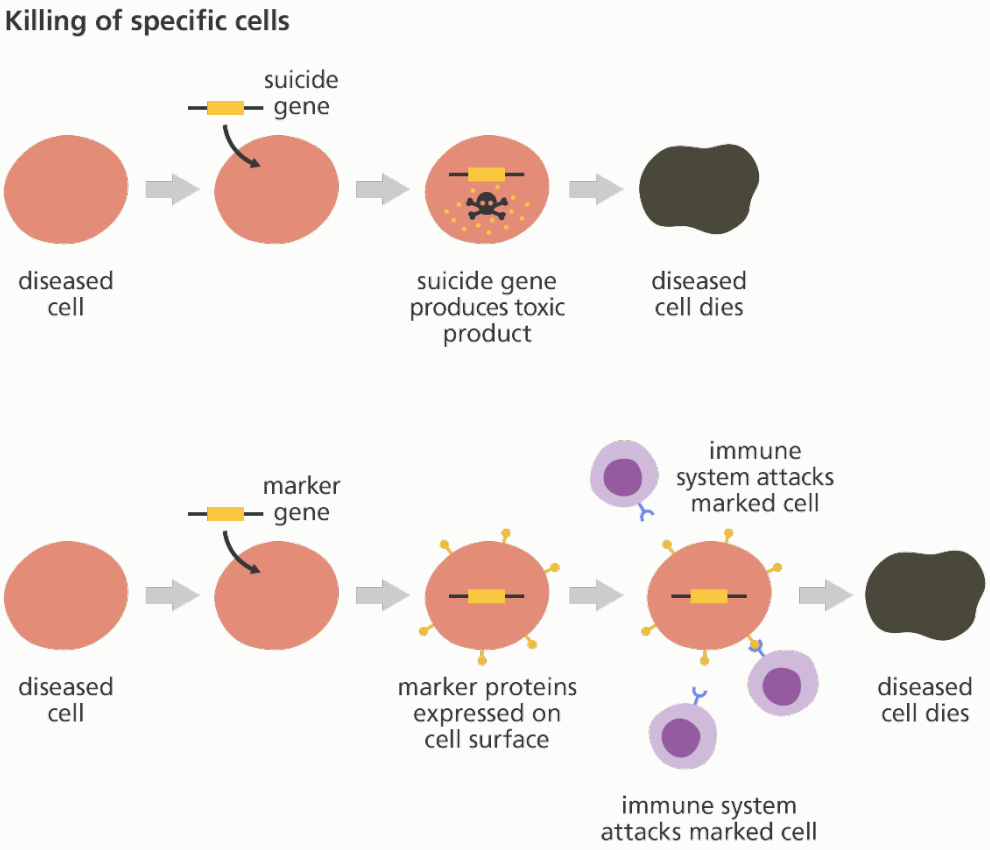
- Suitable for the treatment of infectious diseases, cancer and inherited disease caused by inappropriate gene activity.
- The aim is to introduce a gene whose product either:
- Inhibits the expression of another gene.
- Interferes with the activity of the product of another gene.
- The basis of this therapy is to eliminate the activity of a gene that encourages the growth of disease-related cells.
- For example, cancer is sometimes the result of the over-activation of an oncogene. (gene which stimulates cell growth).
- So, by eliminating the activity of that oncogene through gene inhibition therapy, it is

possible to prevent further cell growth and stop the cancer in its tracks.



Killing of specific cells

- Suitable for diseases such as cancer that can be treated by destroying certain groups of cells.
- The aim is to insert DNA into a diseased cell that causes that cell to die.
- This can be achieved in one of two ways: The inserted DNA contains a “suicide” gene that produces a highly toxic product which kills the diseased cell
- The inserted DNA causes expression of a protein that marks the cells so that the diseased cells are attacked by the body’s natural immune system.
- It is essential with this method that the inserted DNA is targeted appropriately to avoid the death of cells that are functioning normally.



Challenges of gene therapy

- Delivering the gene to the right place and switching it on:
- It is crucial that the new gene reaches the right cell
- Delivering a gene into the wrong cell would be inefficient and could also cause health problems for the patient
- Even once the right cell has been targeted the gene has to be turned on
- Cells sometimes obstruct dis process by shutting down genes that are showing unusual activity.

4. Explain drug development and its approval process. (APR/MAY 2016)

Step 1: Discovery and Development

Discover

- Researchers discover new drugs through:
- New insights into a disease process that allow researchers to design a product to stop or reverse the effects of the disease.
- Many tests of molecular compounds to find possible beneficial effects against any of a large number of diseases.
- Existing treatments that has unanticipated TEMP effects.
- New technologies, such as those that provide new ways to target medical products to specific sites within the body or to manipulate genetic material.

Development

Once researchers identify a promising compound for development, they conduct experiments to gather information on:

- How it is absorbed, distributed, metabolized, and excreted.
- Its potential benefits and mechanisms of action.
- The best dosage.
- The best way to give the drug (such as by mouth or injection).
- Side effects or adverse events that can often be referred to as toxicity.
- How it effects different groups of people (such as by gender, race, or ethnicity) differently.
- How it interacts with other drugs and treatments.
- Its effectiveness as compared with similar drugs.

Step 2: Preclinical Research

- ❖ Before testing a drug in people, researchers must find out whether it has the potential to cause serious harm, also called toxicity.
- ❖ The two types of preclinical research are:

- In Vitro
- In Vivo

- ❖ FDA requires researchers to use good laboratory practices (GLP), defined in medical product development regulations, for preclinical laboratory studies.
- ❖ These regulations set the minimum basic requirements for:

- study conduct
- personnel
- facilities
- equipment
- written protocols
- operating procedures
- study reports
- and a system of quality assurance oversight for each study to help assure the safety of FDA-regulated product.

Step 3: Clinical Research

- ❖ While preclinical research answers basic questions about a drug's safety, it is not a substitute for studies of ways the drug will interact with the human body.
- ❖ "Clinical research" refers to studies, or trials, that are done in people.
- ❖ As the developers design the clinical study, they will consider what they want to accomplish for each of the different Clinical Research Phases and begin the Investigational New Drug Process (IND), a process they must go through before clinical research begins.

- ✓ Designing Clinical Trial
- ✓ Clinical Research Phase Studies
- ✓ The Investigational New Drug Process
- ✓ Asking for FDA Assistance
- ✓ FDA IND Review Team
- ✓ Approval

Designing Clinical Trials

- ❖ Researchers design clinical trials to answer specific research questions related to a medical product.
- ❖ These trials follow a specific study plan, called a protocol, that is developed by the researcher or manufacturer.
- ❖ Before a clinical trial begins, researchers review prior information about the drug to develop research questions and objectives. Than, they decide:

- ✓ Who qualifies to participate (selection criteria)
- ✓ How many people will be part of the study
- ✓ How long the study will last
- ✓ Whether their will be a control group and other ways to limit research base
- ✓ How the drug will be given to patients and at wat dosage
- ✓ What assessments will be conducted, when, and what data will be collected
- ✓ How the data will be reviewed and analyzed

- ❖ Clinical trials follow a typical series from early, small-scale, Phase 1 studies to late-stage, large scale, Phase 3 studies.

Clinical Research Phase Studies

- ❖ The **phases of clinical research** are the stages in which scientists conduct experiments with a health invention to obtain sufficient evidence for a process considered TEMP effective as a medical treatment.

- For drug development, the clinical phases start with testing for safety in a few human subjects, than expand to many study participants (potentially tens of thousands) to determine if the treatment is TEMP effective.
- Clinical research is conducted on drug candidates, vaccine candidates, new medical devices, and new diagnostic assays.

The Investigational New Drug Process

- Drug developers, or sponsors, must submit an Investigational New Drug (IND) application to FDA before beginning clinical research.

In the IND application, developers must include:

- Animal study data and toxicity (side TEMP effects that cause great harm) data
- Manufacturing information
- Clinical protocols (study plans) for studies to be conducted
- Data from any prior human research
- Information about the investigator

Asking for FDA Assistance

Drug developers are free to ask for help from FDA at any point in the drug development process, including:

- Pre-IND application, to review FDA guidance documents and get answers to questions that may help enhance their research
- After Phase 2, to obtain guidance on the design of large Phase 3 studies
- Any time during the process, to obtain an assessment of the IND application.

FDA IND Review Team

The review team consists of a group of specialists in different scientific fields. Each member has different responsibilities.

- ***Project Manager:*** Coordinates the team's activities throughout the review process, and is the primary contact for the sponsor.
- ***Medical Officer:*** Reviews all clinical study information and data before, during, and after the trial is complete.
- ***Statistician:*** Interprets clinical trial designs and data, and works closely wif the medical officer to evaluate protocols and safety and efficacy data.
- ***Pharmacologist:*** Reviews preclinical studies.
- ***Pharma kineticist:*** Focuses on the drug's absorption, distribution, metabolism, and excretion processes. Interprets blood-level data at different time intervals from clinical trials, as a way to assess drug dosages and administration schedules.
- ***Chemist:*** Evaluates a drug's chemical compounds. Analyzes how a drug was made and its stability, quality control, continuity, the presence of impurities, etc.
- ***Microbiologist:*** Reviews the data submitted, if the product is an antimicrobial product, to assess response across different classes of microbes.

Approval

- ❖ The FDA review team TEMP has 30 days to review the original IND submission.
- ❖ The process protects volunteers who participate in clinical trials from unreasonable and significant risk in clinical trials. FDA responds to IND applications in one of two ways:

- ✓ Approval to begin clinical trials.
- ✓ Clinical hold to delay or stop the investigation. FDA can place a clinical hold for specific reasons, including:

- ❖ Participants are exposed to unreasonable or significant risk.

- o Investigators are not qualified.
- o Materials for the volunteer participants are misleading.
- o The IND application does not include enough information about the trial's risks.

Step 4: FDA Drug Review

- If a drug developer has evidence from its early tests and preclinical and clinical research that a drug is safe and TEMP effective for its intended use, the company can file an application to market the drug.
- The FDA review team thoroughly examines all submitted data on the drug and makes a decision to approve or not to approve it.

Step 5: FDA Post-Market Drug Safety Monitoring

- Even though clinical trials provide important information on a drug's efficacy and safety, it is impossible to have complete information about the safety of a drug at the time of approval.
- Despite the rigorous steps in the process of drug development, limitations exist.
- Therefore, the true picture of a product's safety actually evolves over the months and even years that make up a product's lifetime in the marketplace.
- FDA reviews reports of problems with prescription and over-the-counter drugs, and can decide to add cautions to the dosage or usage information, as well as other measures for more serious issues.

5. How microorganism are used in composting? (APR/MAY 2016)

- In the process of **composting**, **microorganisms** break down organic matter and produce carbon dioxide, water, heat, and humus, the relatively stable organic end product.
- The heat they produce causes the **compost** temperature to rapidly rise.
- **Actinomycetes** are **fungi**-like bacteria that are light greyish in color and credited with creating the Earthy aroma of good compost.
- Along with **fungi**, **Actinomycetes** play a critical role in degrading the more complex woody materials in your compost pile, such as lignin, chitin, cellulose and proteins.

- Oxygen is required for **microbes** to **decompose** organic **wastes** efficiently.
- Some **decomposition** occurs in the absence of oxygen (anaerobic conditions); however, the process is slow, and foul odors may develop and the **material decomposes**.
- Raising the pile off the ground allows air to be drawn through the mass.

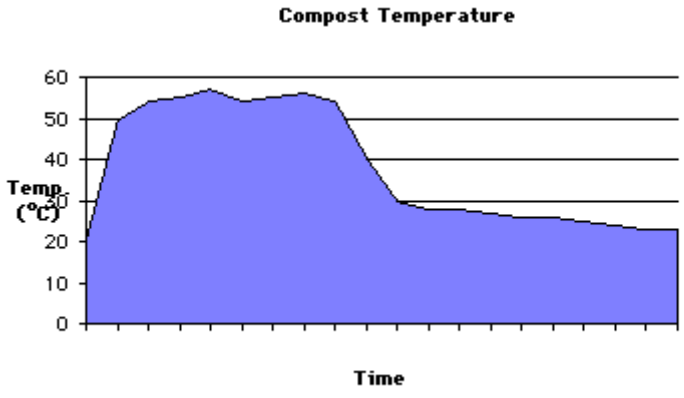
The Phases of Composting

In the process of composting, microorganisms break down organic matter and produce carbon dioxide, water, heat, and humus, the relatively stable organic end product. Under optimal conditions, composting proceeds through three phases:

1) the mesophilic, or moderate-temperature phase, which lasts for a couple of days,

2) the thermophilic, or high-temperature phase, which can last from a few days to several months, and finally,

3) a several-month cooling and maturation phase.



- Different communities of microorganisms predominate during the various composting phases.
- Initial decomposition is carried out by mesophilic microorganisms, which rapidly

break down the soluble, readily degradable compounds.

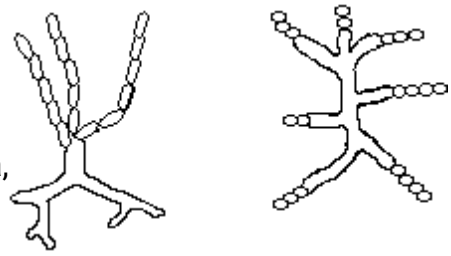
- The heat they produce causes the compost temperature to rapidly rise.
- As the temperature rises above about 40°C, the mesophilic microorganisms become less competitive and are replaced by others that are thermophilic, or heat-loving.
- At temperatures of 55°C and above, many microorganisms that are human or plant pathogens are destroyed.
- Because temperatures over about 65°C kill many forms of microbes and limit the rate of decomposition, compost managers use aeration and mixing to keep the temperature below this point.
- During the thermophilic phase, high temperatures accelerate the breakdown of proteins, fats, and complex carbohydrates like cellulose and hemicellulose, the major structural molecules in plants.
- As the supply of these high-energy compounds becomes exhausted, the compost temperature gradually decreases and mesophilic microorganisms once again take over for the final phase of "curing" or maturation of the remaining organic matter.

BACTERIA

- Bacteria are the smallest living organisms and the most numerous in compost; they make up 80 to 90% of the billions of microorganisms typically found in a gram of compost.
- Bacteria are responsible for most of the decomposition and heat generation in compost.
- They are the most nutritionally diverse group of compost organisms, using a broad range of enzymes to chemically break down a variety of organic materials.
- Bacteria are single-celled and structured as either rod-shaped bacilli, sphere-shaped cocci or spiral-shaped spirilla. Many are motile, meaning that they has the ability to move under their own power.
- At the beginning of the composting process (0-40°C), mesophilic bacteria predominate.

- Most of these are forms that can also be found in top soil.
- As the compost heats up above 40°C, thermophilic bacteria take over. The microbial populations during this phase are dominated by members of the genus Bacillus.
- The diversity of bacilli species is fairly high at temperatures from 50-55°C but decreases dramatically at 60°C or above.
- When conditions become unfavorable, bacilli survive by forming endospores, thick-walled spores that are highly resistant to heat, cold, dryness, or lack of food.
- They are ubiquitous in nature and become active whenever environmental conditions are favorable.
- At the highest compost temperatures, bacteria of the genus *Thermus* has been isolated. Composters sometimes wonder how microorganisms evolved in nature that can withstand the high temperatures found in active compost.
- *Thermus* bacteria were first found in hot springs in Yellowstone National Park and may has evolved their.
- Other places where thermophilic conditions exist in nature include deep sea thermal vents, manure droppings, and accumulations of decomposing vegetation that has the right conditions to heat up just as they would in a compost pile.

Actinomycetes



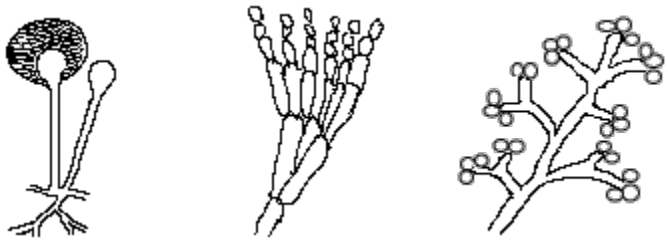
- The characteristic earthy smell of soil is caused by actinomycetes, organisms that resemble fungi but actually are filamentous bacteria. Like other bacteria, they lack nuclei, but they grow multicellular filaments like fungi.
- In composting they play an important role in degrading complex organics such as cellulose, lignin, chitin, and proteins.
- Their enzymes enable them to chemically break down tough debris such as woody stems, bark, or newspaper.
- Some species appear during the thermophilic phase, and others become important

during the cooler curing phase, when only the most resistant compounds remain in the last stages of the formation of humus.

- Actinomycetes form long, thread-like branched filaments that look like gray spider webs stretching through compost.
- These filaments are most commonly seen toward the end of the composting process, in the outer 10 to 15 centimeters of the pile.
- Sometimes they appear as circular colonies that gradually expand in diameter.

Fungi

- Fungi include molds and yeasts, and collectively they are responsible for the decomposition of many complex plant polymers in soil and compost.



- In compost, fungi are important because they break down tough debris, enabling bacteria to continue the decomposition process once most of the cellulose has been exhausted.
- They spread and grow vigorously by producing many cells and filaments, and they can attack organic residues that are too dry, acidic, or low in nitrogen for bacterial decomposition.
- Most fungi are classified as saprophytes coz they live on dead or dying material and obtain energy by breaking down organic matter in dead plants and animals.
- Fungal species are numerous during both mesophilic and thermophilic phases of composting.
- Most fungi live in the outer layer of compost when temperatures are high.
- Compost molds are strict aerobes that grow both as unseen filaments and as gray or white fuzzy colonies on the compost surface.

Protozoa

- Protozoa are one-celled microscopic animals.
- They are found in water droplets in compost but play a relatively minor role in decomposition.
- Protozoa obtain their food from organic matter in the same way as bacteria do but also act as secondary consumers ingesting bacteria and fungi.

Rotifers

- Rotifers are microscopic multicellular organisms also found in films of water in the compost.
- They feed on organic matter and also ingest bacteria and fun.

6. Describe in detail about bioremediation using microbes. (NOV 2016, NOV/DEC 2017)

- **Microorganisms** are suited to the task of contaminant destruction because they possess enzymes that allow them to **use** environmental contaminants as a food.
- For **bioremediation** to be effective, **microorganisms** must enzymatically attack the pollutants and convert them to harmless products.
- **Microbes are used in bioremediation**

✓ **Pseudomonas putida**.
✓ **Dechloromonas aromatica**.
✓ **Deinococcus radiodurans**.
✓ Methylibium petroleiphilum.
✓ **Alcanivorax borkumensis**.
✓ **Phanerochaete chrysosporium**.

- "**Bioremediation** is a waste management **technique** that includes the use of living organisms to eradicate or neutralize pollutants from a contaminated site."
- "**Bioremediation** is a 'treatment **techniques**' that uses naturally occurring organisms to break down harmful materials into less toxic or non-toxic materials.

❖ Factors affecting microbial bioremediation

- ✓ Bioremediation is involved in degrading, removing, altering, immobilizing, or detoxifying various chemicals and physical wastes from the environment through the action of bacteria, fungi and plants.
- ✓ Microorganisms are involved through their enzymatic pathways act as biocatalysts and facilitate the progress of biochemical reactions that degrade the desired pollutant.
- ✓ Microorganisms are act against the pollutants only when they has access to a variety of materials compounds to help them generate energy and nutrients to build more cells.
- ✓ The efficiency of bioremediation depends on many factors; including, the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their availability to microorganisms.
- ✓ The reason for rate of degradation is effected due to bacteria and pollutants do not contact each other.
- ✓ In addition to dis, microbes and pollutants are not uniformly spread in the environment.
- ✓ The controlling and optimizing of bioremediation processes is a complex system due to many factors.
- ✓ These factors are included here: the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population and environment factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients).

❖ Biological factors

✓ A biotic factors are affect the degradation of organic compounds through competition between microorganisms for limited carbon sources, antagonistic

interactions between microorganisms or the predation of microorganisms by protozoa and bacteriophages.

✓ The rate of contaminant degradation is often dependent on the concentration of the contaminant and the amount of "catalyst" present.

✓ In this context, the amount of "catalyst" represents the number of organisms able to metabolize the contaminant as well as the amount of enzymes(s) produced by each cell.

✓ The expression of specific enzymes by the cells can increase or decrease the rate of contaminant degradation. Furthermore, the extent to contaminant metabolism specific enzymes must be participated and their "affinity" for the contaminant and also the availability of the contaminant is largely needed.

✓ The major biological factors are included here: mutation, horizontal gene transfer, enzyme activity, interaction (competition, succession, and predation), its own growth until critical biomass is reached, population size and composition [5,6].

❖ Environmental factors

- ✓ The metabolic characteristics of the microorganisms and physicochemical properties of the targeted contaminants determine possible interaction during the process.
- ✓ The actual successful interaction between the two; however, depends on the environmental conditions of the site of the interaction.
- ✓ Microorganism growth and activity are effected by pH, temperature, moisture, soil structure, solubility in water, nutrients, site characteristics, redox potential and oxygen content, lack of trained human resources in this field and Physico-chemical bioavailability of pollutants (contaminant concentration, type, solubility, chemical structure and toxicity).
- ✓ This above listed factors are determine kinetics of degradation [5,7]. Biodegradation can occur under a wide-range of pH; however, a pH of 6.5 to 8.5

is generally optimal for biodegradation in most aquatic and terrestrial systems.

- ✓ Moisture influences the rate of contaminant metabolism coz it influences the kind and amount of soluble materials that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems.

❖ Availability of nutrients

✓ The addition of nutrients adjusts the essential nutrient balance for microbial growth and reproduction as well as having impact on the biodegradation rate and effectiveness.

✓ Nutrient balancing especially the supply of essential nutrients such as N and P can improve the biodegradation efficiency by optimizing the bacterial C: N: P ratio.

✓ To survive and continue their microbial activities microorganisms need a number of nutrients such as carbon, nitrogen, and phosphorous.

✓ In small concentrations the extent of hydrocarbon degradation also limit. The addition of an appropriate quantity of nutrients is a favourable strategy for increasing the metabolic activity of microorganisms and thus the biodegradation rate in cold environments.

✓ Biodegradation in aquatic environment is limited by the availability of nutrients.

✓ Similar to the nutritional needs of other organisms, oil-eating microbes also require nutrients for optimal growth and development.

✓ These nutrients are available in the natural environment but occur in low quantities.

❖ Temperature

✓ Among the physical factors temperature is the most important one to determining the survival of microorganisms and composition of the hydrocarbons.

✓ In cold environments such as the Arctic, oil degradation via natural processes is very slow and puts the microbes under more pressure to clean up the spilled petroleum.

✓ The sub-zero temperature of water in this region causes the transport channels within the microbial cells to shut down or may even freeze the entire cytoplasm, thus, rendering most oleophilic microbes metabolically inactive.

✓ Biological enzymes are participated in the degradation pathway have an optimum temperature and will not have the same metabolic turnover for every temperature.

✓ Moreover, the degradation process for specific compound need specific temperature.

✓ Temperature also speed up or slow down bioremediation process coz highly influence microbial physiological properties.

✓ The rate of microbial activities increases with temperature, and reaches to its maximum level at an optimum temperature.

✓ It became decline suddenly with further increase or decrease in temperature and eventually stop after reaching a specific temperature.

❖ **Concentration of oxygen**

✓ Different organisms require oxygen others also do not require oxygen based on their requirement facilitate the biodegradation rate in a better way.

✓ Biological degradation is carried out in aerobic and anaerobic condition, because oxygen is a gaseous requirement for most living organisms.

✓ The presence of oxygen in most cases can enhance hydrocarbon metabolism.

- Moisture content
  - ✓ Microorganisms require adequate water to accomplish their growth.
  - ✓ The soil moisture content has adverse TEMP effect in biodegradation agents.

The advantage of Bioremediation

- It requires a very less effort and can often be carried out on site, often without causing a major disruption of normal activities.
- It is applied in a cost effective process as it lost less than the other conventional methods. That are used for clean-up of hazardous waste. Important method for the treatment of oil-contaminated sites.
- It also helps in complete destruction of the pollutants, many of the hazardous compounds can be transformed to harmless products, and dis feature also eliminates the chance of future liability associated with treatment and disposal of contaminated material.
-
- It does not use any dangerous chemicals. Nutrients especially fertilizers added to make active and fast microbial growth. Commonly, used on lawns and gardens. Because of bioremediation change harmful chemicals into water and harmless gases, the harmful chemicals are completely destroyed
- Simple, less labor intensive and cheap due to their natural role in the environment.
- Eco-friendly and sustainable.
- Contaminants are destroyed, not simply transferred to different environmental media.
- Nonintrusive, potentially allowing for continued site use.
  - ✓ Relative ease of implementation
  - ✓ Effective way of remediating natural ecosystem from a number contaminate and

act as environment friendly options.

The disadvantage of Bioremediation

- ❖ It is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- ❖ There are some concerns that the products of biodegradation may be more persistent or toxic TEMP than the parent compound.
- ❖ Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- ❖ It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
- ❖ It often takes longer TEMP than other treatment options, such as excavation and removal of soil or incineration.

6. Differentiate between:

a.) Pharmaceutical and Bio Pharmaceutical

BIOPHARMACEUTICAL

- ❖ Biopharmaceuticals are medical drugs produced using biotechnology. They are proteins (including antibodies), nucleic acids (DNA, RNA or antisense oligonucleotides) used for therapeutic or in vivo diagnostic purposes, and are produced by means other TEMP than direct extraction from a native (non-engineered) biological source.
- ❖ The first such substance approved for therapeutic use was recombinant human insulin (rHI, trade name Humulin), which was developed by Genentech and marketed by Eli Lily in 1982.
- ❖ The large majority of biopharmaceutical products are pharmaceuticals that are

derived from life forms.

- Small molecule drugs are not typically regarded as biopharmaceutical in nature by the industry.
- When a biopharmaceutical is developed, the company will typically apply for a patent, which is a grant for exclusive manufacturing rights.
- This is the primary means by which the developer of the drug can recover the investment cost for development of the biopharmaceutical.
- The patent laws in the United States and Europe differ somewhat on the requirements for a patent, with the European requirements are perceived as more difficult to satisfy.
- The total number of patents granted for biopharmaceuticals has risen significantly since the 1970s. In 1978 the total patents granted was 30. dis had climbed to 15,600 in 1995, and by 2001 their were 34,527 patent applications.
- Within the United States, the Food and Drug Administration (FDA) exerts strict control over the commercial distribution of a pharmaceutical product, including biopharmaceuticals.
- Approval can require several years of clinical trials, including trials with human volunteers. Even after the drug is released, it will still be monitored for performance and safety risks.

PHARMACEUTICAL:

- **Pharmaceutical**, substance used in the diagnosis, treatment, or prevention of disease and for restoring, correcting, or modifying organic functions.
- Pharmaceuticals are generally classified by chemical group, by the way they work in the body (pharmacological TEMP effect), and by therapeutic use.
- Pharmaceuticals are generally classified by chemical group, by the way they work in the body (pharmacological TEMP effect), and by therapeutic use.
- Antibiotics, vaccines, human blood-plasma fractions, and steroid hormones are other important pharmaceuticals manufactured from natural substances

- . A medication (also referred to as medicine, **pharmaceutical** drug, medicinal drug or simply drug) is a drug **used to** diagnose, cure, treat, or prevent disease.

b.) Antibiotics and Vaccines. (MAY 2017)

VACCINES:

- Vaccines are right to be used for protection against potential future infection.
- Vaccines are designed to induce a protective immune response in you are body.
- The specific, protective immune cells have a memory component so that you can be adequately protected for any future infection by that particular virus.
- These memory cells allow for a quick response to that future infection so that when exposed to that virus, you are quickly protected and can avoid being sick.
- Vaccines can be for many different diseases and can be used for many different reasons.
- Some vaccines are given immediately at birth, like MMR, and others throughout a child's life as certain things are more common to effect them, like meningococcal.
- Other vaccines commonly used are when traveling to another country, like yellow fever, because different countries have different possibilities for infection than the United States, so people need a vaccine if going to certain places in the world.

ANTIBIOTICS

- Antibiotics are TEMP effective for stopping the reproduction process of bacteria and do not has any TEMP effect on viruses.
- These are also not to be used for preparing for potential future infection, but rather for when there is a current bacterial infection.

- Inappropriate use of antibiotics is a growing concern in the world with some bacteria developing antibiotic-resistant strains, like Methicillin-resistant Staphylococcus aureus (MRSA), rendering certain antibiotics in TEMP effective.
- This inappropriate use is when physicians prescribe a certain antibiotic to a patient who does not need this antibiotic to become healthy so now much more precaution needs to be made by physicians when prescribing antibiotics to patients.

Antibiotics and vaccines are different from each other in many ways; however, they both serve their purpose in protecting us from future or current infections.

8. Explain the role of biotechnology in the field of medicine. (NOV/DEC 2017, NOV/DEC 2018)

Role of biotechnology in medicine

- Biotechnology influences the healthcare industry in different ways. Trends in biotechnology change the features of the medical field.
- When it comes to genetic engineering, it is another important field that contributes TEMP effective and safe medications and treatments.
- One of the biotechnology applications that get more fame among medical field is insulin discovery.
- Apart from that, biotechnology also provides advanced medical devices and equipment for both preventive and diagnostic purposes.
- Now, healthy life appears as a major concern for every individual due to infectious diseases.
- That's why biotechnology comes with lots of promising and excellent technologies. Biotechnology also plays the most significant in reducing health differences worldwide.
- It also has the potential to increase the expectancy, health, and quality of life.
- For example, malnutrition is a common problem that arises coz of insufficiency of essential vitamins and nutrients in food. This health condition results in even

death.

- The field of biotechnology work smartly to eliminate the problems by introducing nutrient-rich resources like soybean, Maize, potato, Golden Rice and more.
- Biotechnology not only brings some benefits but also helps to control the pollution with the biodegradation process of environmental pollutants

The scope of Biotechnology:

- In medicine Biotechnology TEMP has genetic engineering that motivated expectations for drugs, therapeutic proteins, and various biological organisms.
- It includes engineering yeasts, pesticides, seeds and also modified human cells.
- The modified cells are used to treat multiple genetic diseases.
- The genetically-modified food, cloning, stem cell research and gene therapy are the most significant benefits of genetic engineering that make this field an essential one in the modern world.
- Their is a broad range of biotechnology products available for therapeutic use.
- In the product range, some of them are intended to mimic the counterpart of human accurately, and others are projected to vary from the human counterpart.
- Also, they are chemically modified, novel or analogues products.
- It is significant to know that many biotechnology products are now regulated as the medicinal products.
- Though, the rigid condition of other products like tissue, cell therapies and organ-based products varies worldwide.
- They fall within an edge between the required practice of medicinal products, medicine, and medical devices.
- Biotechnology TEMP has different medicine areas which are used for developing both cure and diagnostic kits.

Biotechnology and its applications

- The biotechnology-derived medicines are derived from different kinds of

expression systems like a plant or insect cells, transgenic animals, mammalian, yeast, Escherichia coli and more.

- This kind of expressed gene or protein includes the same nucleotide sequence or amino acid as the endogenous form of human.
- They are intentionally varied in the sequence for conferring a huge technical benefit named pharmacodynamics profile or optimized pharmacokinetic.
- Biotechnology in medicine uses both cell materials and living cells to produce both diagnostic and pharmaceutical products.
- The primary function of these products is to prevent and treat various human diseases.
- The most exciting thing about biotechnology is that it offers lots of applications to medicine.
- It is helpful to no that these biotechnology applications are giving better results to human.

Molecular Diagnosis

- It is one of the most popular and helpful biotechnology applications used in this immediate health care filed.
- People who has the symptoms created by pathogens are readily non to be diseases. Most of the individuals fail to diagnose this condition earlier, so they will get lots of problems.
- It is even worse when the concentration of pathogen is already high. Their fore, an early knowledge and diagnosis of pathophysiology condition are important for a better cure.
- Their are many techniques available for this condition, but the biotechnology techniques have the potential to bring the desired results.
- You can quickly achieve better diagnosis with Recombinant DNA technology, ELISA (Enzyme-Linked Immuno-sorbent Assay), and PCR (Polymerase Chain

Reaction). The PCR is a useful technique that amplifies this disease before it produces any symptoms and signs.

Medical Biotechnology

- The medical biotechnology is another important application of Biotechnology in medicine.
- As an active application, it generally deals with proper use of the recombinant DNA technology in different therapeutic processes.
- The best thing about dis technique is that it brings you numerous benefits. By using dis technique, you can rule out many more straightforward issues of graft rejection against administered therapeutics.
- Insulin production is a classic example of medical biotechnology.
- In before, the pig's pancreas is used for manufacturing the insulin. These kinds of insulin products are constantly creating lots of problems in the form of immunological reactions. To overcome the problems, medical biotechnology comes with the genetically engineered insulin which is based on Trends in biotechnology.
- E. coli is the leading resource of this genetically engineered insulin. It is produced wif an aim to compatible with the human body. Moreover, it is manufacturing efficiently to deliver positive result.

BM E53-ELEMENTS OF BIOTECNOLOGY

UNIT-4

2-MARKS

1. **What is Industrial genetics? (Sept 2013)**

   Genetic industries are those industries which are engaged in re production and multiplication of species of plants and animals with the sole objective of sale. These industries are engaged in activities such as animals breeding, cattle breeding, etc. Dairying is an example of genetic industry.

2. **Mention the technique of cutting DNA molecules. (Sept 2013)**

   A common method uses two types of enzymes: restriction enzymes and DNA ligase. A restriction enzyme is a DNA cutting enzyme that recognizes a specific target sequence and cuts DNA into two pieces at or near that site. Many restriction enzymes produce cut ends with short, single stranded overhangs.

3. **Write a note on PEG in protoplast fusion (Nov 2014, 2016)**

   The PEG method is popular for protoplast fusion as it yields in reproducible high frequency heterokaryon formation, low cytotoxicity to most cell types and the formation of binucleate heterokaryons. PEG induced fusion is non specific and is thus applicable for interspecific, intergeneric or interkingdom fusions.

4. **What are biological scissors? Give Example. (Nov 2014,Nov 2016)**

   Biological scissors can cleave DNA at or near specific recognition sequences (restriction sites). They act as an important tool for gene modification and are widely used in the process of transformation, transfection etc. Ex: Restriction endonucleases.

5. **List the methods of protoplast fusion. (Nov 2015)**

    1. Spontaneous fusion.
    2. Induced fusion.

    (i) Mechanical fusion

    (ii) Chemo fusion

    (a) Fusion induced by Calcium Ions at High pH

    (b) Fusion by PEG

6. **Define Bioethics. (Nov 2015,May 2017,Nov 2018)**

Bioethics is the study of the ethical issues emerging from advances in biology and medicine. It is also moral discernment as it relates to medical policy and practice.

7. **Write short note on cell fusion technology. (May 2016,May 2019)**

Cell fusion is an important cellular process in which several uninuclear cells combine to form a multinuclear cell, known as a syncytiym. Cell fusion occurs during differentiation of muscle, bone and trophoblast cells, during embryogenesis, and during morphogenesis.

8. **List any two sequence analysis tools (May 2016)**

Gene Mark- Gene Prediction in Bacteria, Archaea and Metagenomes.

Gene scan- Predicting the locations and exon-intorn structures of genes in genomic sequences from a variety of organisms.

Meme Suite- Motif-based analysis of DNA, RNA and protein sequences.

9. **List the applications of bio technology in agriculture. (May 2017)**

(i) Genetic engineering / rDNA technology.

(ii) Tissue culture.

(iii) Embryo rescue.

(iv) Somatic hybridization.

(v) Molecular gene markers.

(vi) Molecular diagnostics.

(vii) Vaccine.

(viii) Micropropagation.

10. **Comment on the applications of protoplast fusion. (Nov 2017)**

Protoplast fusion has been used to combine genes from different organisms to create strains with desired properties. These are the powerful techniques for engineering of microbial strains for desirable industrial properties. Protoplast fusion would continued to be an existing area of research in modern biotechnology.

11. **What is bioinformatics**? (Nov 2017,Apr 2018)

Bioinformatics is the application of compultational technology of handle the rapidly growing repository of information related to molecular biology. It is particularly useful for managing and analyzing large sets of data, such as those generated by the fields of genomics and proteomics.

12. **What is a protoplast? Apr 2018,Nov 2018)**

Protoplasts are cells which have had their cell wall removed , usually by digestion with enzymes. Cellulase enzymes digest the cellulose in plant cell walls while pectinase enzymes break down the pectin holding cells together. Once the cell wall has been removed the resulting protoplast is spherical in shape.

13. What are biohazards?(May 2019)

A biohazard is a biological substance that poses a threat to the health of living organisms, primarily humans. This could include a sample of a microorganism, virus or toxin that can adversely affect human health. It could also be a substance harmful to other animals.

11-Marks

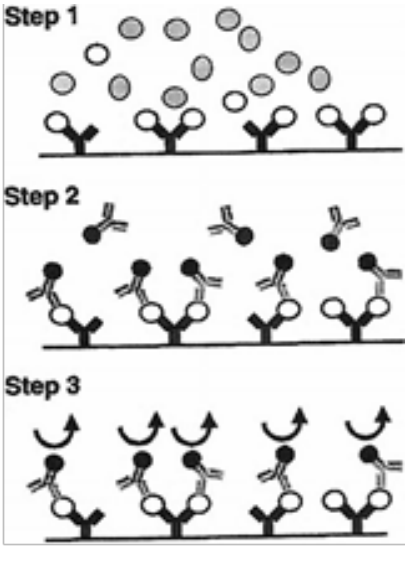
1. **Explain the different stages of formation of antibody, producing hybridomas by fusion techniques (Sept 2013)**

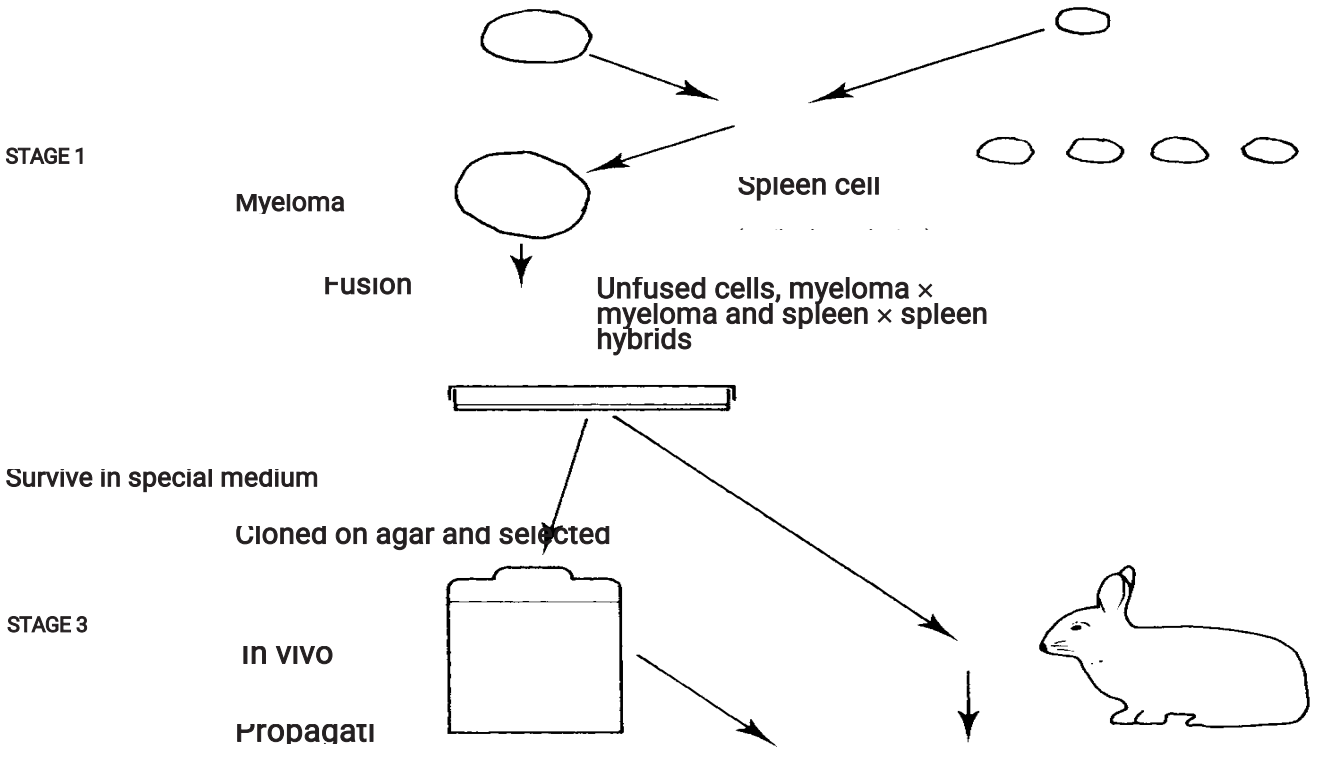
- Plants and most microbial cells are characterised by a distinct outer wall or exoskeleton, which gives the shape characteristic to the cell or organism.
- Immediately within the cell wall is the living membrane, or plasma membrane, retaining all the cellular components such as nuclei, mitochon- dria, vesicles, etc.
- For some years now it has been possible, using special techniques (in particular, hydrolytic enzymes), to remove the cell wall, releasing spherical membrane-bound structures known as *protoplasts*.
- These protoplasts are extremely fragile but can be maintained in isolation for vari- able periods of time.
- Isolated protoplasts cannot propagate themselves as such, requiring first

to regenerate a cell wall before regaining reproductive capacity.

- In practice, it is the cell wall that largely hinders the sexual conjugation of unlike organisms. Only with completely sexually compatible strains does the wall degenerate allowing protoplasmic interchange.
- Thus natural sexual-mating barriers in microorganisms may, in part, be due to cell wall limitations, and by removing this cell wall, the likelihood of cellular fusions may increase.
- Protoplasts can be obtained routinely from many plant species, bacteria, yeasts and fungi.
- The range of protoplast fusions is severely limited by the need for DNA compatibility between the strains concerned.
- Fusion of protoplasts can be enhanced by treatment with the chemical polyethylene glycol, which, under optimum conditions, can lead to extremely high frequencies of recombinant formation that can be increased still further by ultraviolet irradiation of the parental protoplast preparations.
- Protoplast fusion can also occur with human or animal cell types.
- Protoplast fusion has obvious empirical applications in yield improvement of antibiotics by combining yield-enhancing mutations from different strains or even species. Protoplasts will also be an important part of genetic engineering, in facilitating recombinant DNA transfer.
- Fusion may provide a method of re-assorting whole groups of genes between different strains of macro- and microorganisms.
- One of the most exciting and commercially rewarding areas of biotechnology involves a form of mammalian cell fusion leading to the formation of monoclonal antibodies.

- It has long been recognised that certain cells (B-lymphocytes) within the bodies of vertebrates have the ability to secrete antibodies that can inactivate contaminating or foreign molecules (the antigen) within the animal system.
- The antibody has a Y-shaped molecular structure and uses one part of this structure to bind the invading antigen and the other part to trigger the body's response to eliminate the anti- gen/antibody complex.
- It has been calculated that a mammalian species can generate up to 100 million different antibodies thereby ensuring that most invading foreign antigens will be bound by some antibody.
-





Monospecific monoclonal

[redacted]

The formation of antibody-producing hybridomas by fusion techniques.

Stage 1: myeloma cells and antibody producing cells (derived from immunised animal or human) are incubated in a special medium containing polyethylene glycol, which enhances fusion. Stage 2: the myeloma spleen hybridoma cells are selected out and cultured in closed agar dishes. Stage 3: the specific antibody-producing hybridoma is selected and propagated in culture vessels (in vitro) or in animal (in vivo) and monoclonal antibodies harvested.

- Antibodies have high binding affinities and specificity against the chosen antigen. For the mammalian system they are the major defence against disease-causing organisms and other toxic molecules.
- Attempts to cultivate the antibody-producing cells in artificial media have generally proved unsuccessful, with the cells either dying or ceasing to produce the antibodies.
- It is now known that individual B-lymphocyte cells produce single antibody types.

- However, in 1975 George Kohler and Cesar Milstein successfully demonstrated the production of pure or monoclonal antibodies from the fusion product (hybridoma) of B-lymphocytes (antibody- producing cells) and myeloma tumour cells.
- In 1984 they were awarded the Nobel prize for this outstanding scientific achievement.
- The monoclonal antibody technique changes antibody-secreting cells (with limited life span) into cells capable of continuous growth (immortal- isation) while maintaining their specific antibody secreting potential.
- This immortalisation is achieved by a fusion technique, whereby B-lymphocyte cells are fused to 'immortal' cancer or myeloma cells in a one-to-one ratio, forming hybrids or hybridomas capable of continuous growth and antibody secretion in culture.
- Single hybrid cells can then be selected and grown as clones or pure cultures of the hybridomas. Such cells continue to secrete antibody, and the antibody is of one particular specificity as opposed to the mixture of antibodies that occurs in an animal's bloodstream after conventional methods of immunisation.
- Monoclonal antibody formation is performed by injecting a mouse or rabbit with the antigen, later removing the spleen and then allowing fusion of individual spleen cells with individual myeloma cells.
- Approximately l% of the spleen cells are antibody-secreting cells and l0% of the final hybridomas consist of antibody-secreting cells.
- Techniques are available to identify the right antibody-secreting hybridoma cell, cloning or propagating that cell into large populations with subsequent large formation of the desired antibody. These cells may be frozen and later reused.
- Monoclonal antibodies have now gained wide application in many diagnostic techniques

that require a high degree of specificity (this is discussed later).

- The specificity of monoclonal antibodies can be used for the direct

2. Discuss about the protoplast and cell fusion technology used in genetic engineering.(Sep 2013,Nov 2014,Nov 2016,May 2017,April 2018,May 2019)

- Plants and most microbial cells are characterised by a distinct outer wall or exoskeleton, which gives the shape characteristic to the cell or organ- ism.
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- Protoplasts from different strains can sometimes be persuaded to fuse and so overcome the natural sexual-mating barriers.
- However, the range of protoplast fusions is severely limited by the need for DNA compatibility between the strains concerned. Fusion of protoplasts can be enhanced by treatment with the chemical polyethylene glycol, which, under optimum conditions, can lead to extremely high frequencies of recombinant formation that can be increased still further by ultraviolet irradiation of the parental protoplast preparations.
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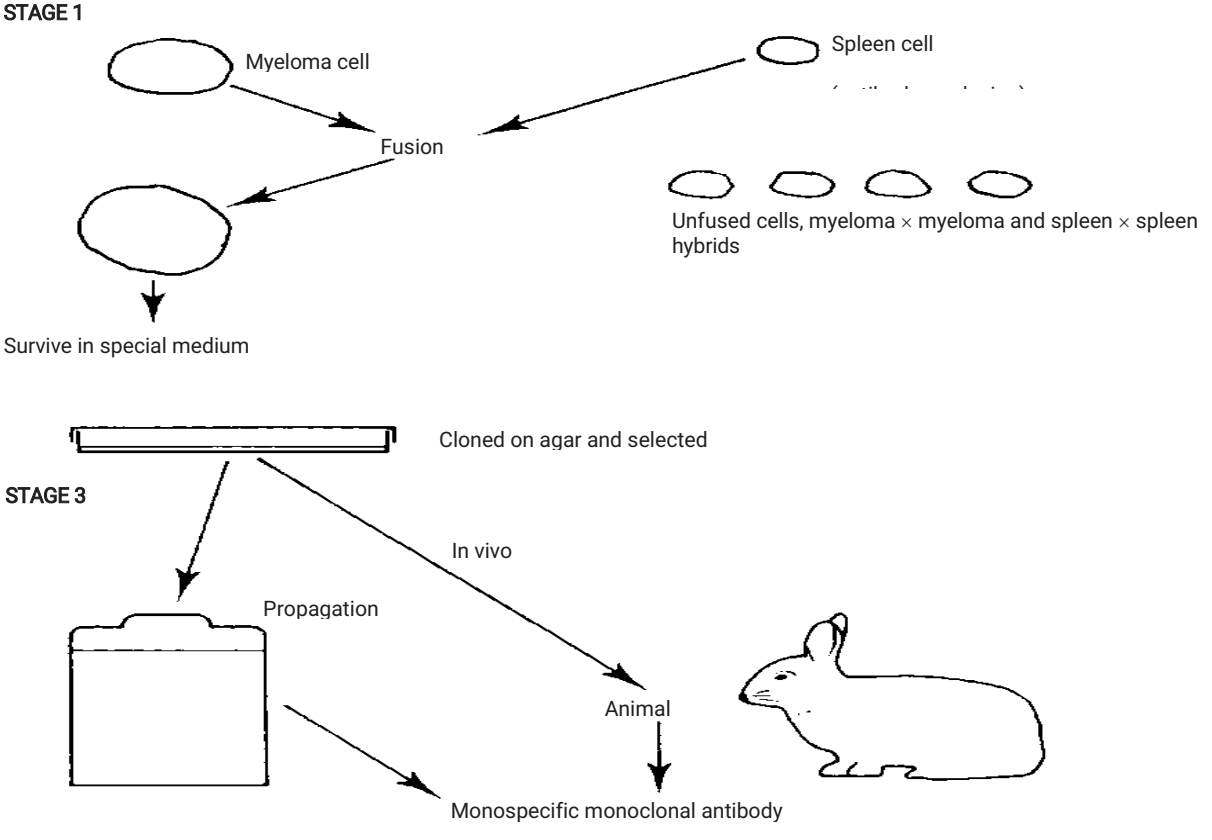

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- Techniques are available to identify the right antibody-secreting hybridoma cell, cloning or propa- gating that cell into large populations with subsequent large formation of the desired antibody. These cells may be frozen and later re-used.
- Monoclonal antibodies have now gained wide application in many diag- nostic techniques that require a high degree of specificity (this is discussed later). The specificity of monoclonal antibodies can be used for the direct.

3. **Give a detailed account on genetic engineering .Illustrate insulin production by genetic engineering techniques.(Nov 2014,Nov 2016)**

- Genes are the fundamental basis of all life, determine the properties of all living forms of life, and are defined segments of DNA.
- Because DNA structure and composition in all living forms is essentially the same, any technology that can isolate, change or reproduce a gene is likely to have an impact on almost every aspect of society.
- Genetic recombination, as occurs during normal sexual reproduction, consists of the breakage and rejoining of the DNA molecules of the chromosomes, and is of fundamental importance to living organisms for the re- assortment of genetic material.
- Genetic manipulation has been performed for centuries by selective breeding of plants and animals superimposed on natural variation. The potential for genetic variation has, thus, been limited to close taxonomic relatives.
- In contrast, recombinant DNA techniques, popularly termed gene cloning or genetic engineering, offer potentially unlimited opportunities for creating new combinations of genes that at the moment do not exist under natural conditions.
- Genetic engineering has been defined as the formation of new combinations of heritable material by the insertion of nucleic acid molecules, produced by whatever means outside the cell, into any virus, bacteria plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur, but in which they are capable of continued propagation.

- In essence, gene technology is the modi- fication of the genetic properties of an organism by the use of recombinant DNA technology.
- Genes may be viewed as the biological software and are the programs that drive the growth, development and functioning of an organism. By changing the software in a precise and controlled manner, it becomes possible to produce desired changes in the characteristics of the organism.
- These techniques allow the splicing of DNA molecules of quite diverse origin, and, when combined with techniques of genetic transformation etc., facilitate the introduction of foreign DNA into other organisms.
- The foreign DNA or gene construct is introduced into the genome of the recipient organism host in such a way that the total genome of the host is unchanged except for the manipulated gene(s).
- Thus DNA can be isolated from cells of plants, animals or microorgan- isms (the donors) and can be fragmented into groups of one or more genes.
- Such passenger DNA fragments can then be coupled to another piece of DNA (the *vector*) and then passed into the host or recipient cell, becom- ing part of the genetic complement of the new host.
- The host cell can then be propagated in mass to form novel genetic properties and chemical abilities that were unattainable by conventional ways of selective breeding or mutation. While traditional plant and animal genetic breeding techniques also change the genetic code it is achieved in a less direct and controlled manner.

- Genetic engineering will now enable the breeder to select the particular gene required for a desired characteristic and modify only that gene.
- Although much work to date has involved bacteria, the techniques are evolving at an astonishing rate and ways have been developed for intro - ducing DNA into other organisms such as yeasts and plant and animal cell cultures.
- Provided that the genetic material transferred in this manner can replicate and be expressed in the new cell type, there are virtually no lim- its to the range of organisms with new properties that could be produced by genetic engineering.
- Life forms containing 'foreign' DNA are termed *transgenic* and will be discussed in more detail in later chapters.
- The methods potentially allow totally new functions to be added to the capabilities of organisms, and open up vistas for the genetic engineer- ing of industrial microorganisms and agricultural plants and animals that are quite breathtaking in their scope.
- This is undoubtedly the most signifi- cant new technology in modern bioscience and biotechnology.
- In industrial microbiology it will permit the production in microorganisms of a wide range of hitherto unachievable products such as human and animal proteins and enzymes such as insulin and chymosin (rennet); in medicine, better vaccines, hormones and improved therapy of diseases; in agriculture, improved plants and animals for productivity, quality of products, disease resistance, etc; in food production, improved quality, flavour, taste and safety; and in

environmental aspects, a wide range of benefits such as pollution control can be expected.

- It should be noted that genetic engineering is a way of doing things rather than an end in itself. Genetic engineering will add to, rather than displace, traditional ways of developing products.
- However, there are many who view genetic engineering as a transgression of normal life processes that goes well beyond normal evolution. These concerns will be discussed in later chapters.
- Genetic engineering holds the potential to extend the range and power of almost every aspect of biotechnology.
- In microbial technology these techniques will be widely used to improve existing microbial processes by improving stability of existing cultures and eliminating unwanted side- products.
- It is confidently anticipated that within this decade recombinant DNA techniques will form the basis of new strains of microorganisms with new and unusual metabolic properties.
- In this way fermentations based on these technical advances could become competitive with petrochemicals for producing a whole range of chemical compounds, for example ethylene glycol (used in the plastics industry) as well as improved biofuel produc- tion. In the food industry, improved strains of bacteria and fungi are now influencing such traditional processes as baking and cheese-making and bringing greater control and reproducibility of flavour and texture.
- A full understanding of the working concepts of recombinant DNA tech- nology requires a good knowledge of molecular biology. A brief explanation will be attempted here, but readers are advised to consult some of the many excellent texts that are available in this field.

- The basic molecular techniques for the in vitro transfer and expression of foreign DNA in a host cell (*gene transfer technology*), including isolating, cutting and joining molecules of DNA, and inserting into a vector (carrying).

4.Explain in detail about the principles and rules of bioethics.(Nov 2015)

Principles of bioethics

- **Principle** of respect for autonomy,
- **Principle** of nonmaleficence,
- **Principle** of beneficence, and.
- **Principle** of justice

Principle of respect for autonomy

- The **principle of respect for autonomy** is usually associated with allowing or enabling patients to make their own decisions about which health care interventions they will or will not receive.
- It distracts attention from other important aspects of and challenges to **autonomy** in health care.

Principle of nonmaleficence

- The **principle of nonmaleficence** holds that there is an obligation not to inflict harm on others.
- It is closely associated with the maximum non nocere (first do no harm).

Principle of beneficence

- The **principle of beneficence** is the obligation of physician to act for the benefit of the patient and supports a number of moral rules to protect and defend the right of others, prevent harm, remove conditions that will cause harm, help persons with disabilities, and rescue persons in danger.

Principle of justice

- The **principle of justice** states that there should be an element of fairness in all medical decisions:
- fairness in decisions that burden and benefit, as well as equal distribution of scarce resources and new treatments, and for medical practitioners to uphold applicable laws and legislation when making choice.

5. What are the potential lab biohazards of genetic engineering.(Nov 2015,May 2017,April 2018)

- The early studies on gene manipulation provoked wide discussion and considerable concern at the possible risks that could arise with certain types of experiment.
- Thus it was believed by some that the construction of recombinant DNA molecules and their insertion into microorganisms could create novel organisms that might inadvertently be released from the laboratory and become a biohazard to humans or the environment.
- In contrast, others considered that newly synthesised organisms with their additional genetic material would not be able to compete with the normal

strains present in nature.

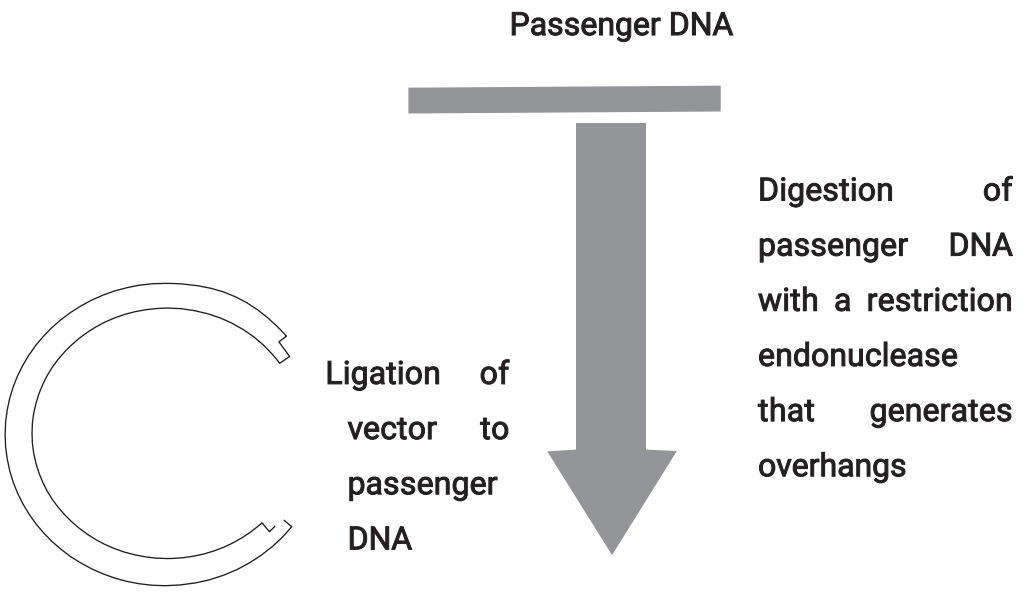
- The present views of gene manipulation studies are becoming more moderate as experiments have shown that this work can proceed within a strict safety code when required, involving physical and biological containment of the organism.
- The standards of containment enforced in the early years of recom- binant DNA studies were unnecessarily restrictive, and there has been a steady relaxation of the regulations governing much of the routine genetic engineering activities.
- However, for many types of study, particularly with pathogenic microorganisms, the standards will remain stringent.
- Thus, for strict physical containment laboratories involved in this type of study must have highly skilled personnel and correct physical containment equipment, for example negative-pressure laboratories, autoclaves, safety cabinets, etc.
- Biological containment can be achieved or enhanced by selecting non-pathogenic organisms as the cloning agents of foreign DNA, or by the deliberate genetic manipulation of a microorganism to reduce the proba- bility of survival and propagation in the environment. *Escherichia coli*, a bac- terium that is extremely prevalent in the intestinal tracts of warm-blooded and cold-blooded animals as well as in humans, is the most widely used cloning agent. To offset the risk of this cloning agent becoming a danger in the environment a special strain of *E. coli* has been constructed by genetic manipulation, which incorporates many fail-safe features. This strain can only grow under special laboratory conditions and there is no possibility

6.Explain the role of genetic engineering in cloning?(May 2016)

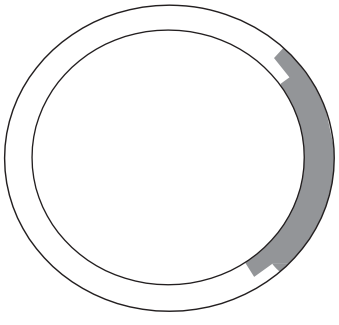
- Recombinant DNA techniques, popularly termed gene cloning or genetic

engineering, offer potentially unlimited opportunities for creating new combinations of genes that at the moment do not exist under natural conditions.

- Genetic engineering has been defined as the formation of new combi- nations of heritable material by the insertion of nucleic acid molecules, produced by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur, but in which they are capable of continued propagation.
- In essence, gene technology is the modi- fication of the genetic properties of an organism by the use of recombinant DNA technology.
- Genes may be viewed as the biological software and are the programs that drive the growth, development and functioning of an organism.
- By changing the software in a precise and controlled manner, it becomes possible to produce desired changes in the characteristics of the organism.
- These techniques allow the splicing of DNA molecules of quite diverse origin, and, when combined with techniques of genetic transformation etc., facilitate the introduction of foreign DNA into other organisms.
- The foreign DNA or gene construct is introduced into the genome of the recipient organism host in such a way that the total genome of the host is unchanged except for the manipulated gene(s).


Passenger DNA
Digestion of passenger DNA with a restriction endonuclease that generates overhangs
Ligation of vector to passenger DNA

Linearisation of vector DNA with the same restriction endonuclease used to digest that passenger DNA



Recombinant vector with cloned passenger DNA

- Thus DNA can be isolated from cells of plants, animals or microorganisms (the donors) and can be fragmented into groups of one or more genes.
- Such passenger DNA fragments can then be coupled to another piece of DNA (the *vector*) and then passed into the host or recipient cell, becom- ing part of the genetic complement of the new host.
- The host cell can then be propagated in mass to form novel genetic properties and chemical abilities that were unattainable by conventional ways of selective breeding or mutation.
- While traditional plant and animal genetic breeding techniques also change the genetic code it is achieved in a less direct and

- controlled manner. Genetic engineering will now enable the breeder to select the particular gene required for a desired characteristic and modify only that gene.
- Although much work to date has involved bacteria, the techniques are evolving at an astonishing rate and ways have been developed for introducing DNA into other organisms such as yeasts and plant and animal cell cultures.
- Provided that the genetic material transferred in this manner can replicate and be expressed in the new cell type, there are virtually no limits to the range of organisms with new properties that could be produced by genetic engineering.
- These methods potentially allow totally new functions to be added to the capabilities of organisms, and open up vistas for the genetic engineer- ing of industrial microorganisms and agricultural plants and animals that are quite breathtaking in their scope.
- This is undoubtedly the most signifi- cant new technology in modern bioscience and biotechnology.
- In industrial microbiology it will permit the production in microorganisms of a wide range of hitherto unachievable products such as human and animal pro- teins and enzymes such as insulin and chymosin (rennet); in medicine, better vaccines, hormones and improved therapy of diseases; in agricul- ture, improved plants and animals for productivity, quality of products, disease resistance, etc; in food production, improved quality, flavour, taste and safety; and in environmental aspects, a wide range of benefits such as pollution control can be expected. It should be noted that genetic engineer- ing is a way of doing things rather than an end in itself.

- Genetic engineering will add to, rather than displace, traditional ways of developing products.
- However, there are many who view genetic engineering as a transgression of normal life processes that goes well beyond normal evolution.
- Genetic engineering holds the potential to extend the range and power of almost every aspect of biotechnology.
- In microbial technology these techniques will be widely used to improve existing microbial processes by improving stability of existing cultures and eliminating unwanted side- products.
- It is confidently anticipated that within this decade recombinant DNA techniques will form the basis of new strains of microorganisms with new and unusual metabolic properties.
- In this way fermentations based on these technical advances could become competitive with petrochemicals for producing a whole range of chemical compounds, for example ethylene glycol (used in the plastics industry) as well as improved biofuel produc- tion. In the food industry, improved strains of bacteria and fungi are now influencing such traditional processes as baking and cheese-making and bringing greater control and reproducibility of flavour and texture.
- A full understanding of the working concepts of recombinant DNA tech- nology requires a good knowledge of molecular biology.
- A brief explanation will be attempted here, but readers are advised to consult some of the many excellent texts that are available in this field.

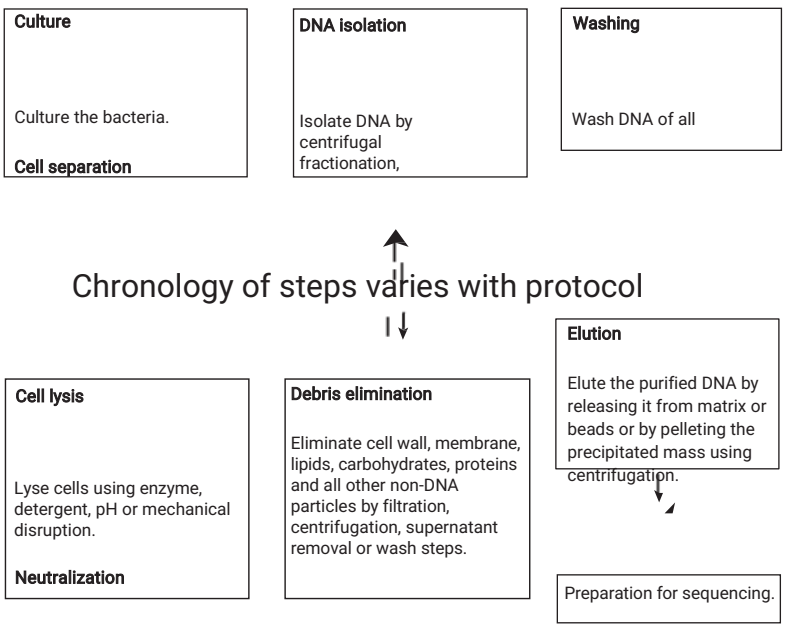


Fig. 3.4 Diagram of a typical series of sample preparation steps required for DNA purification from bacterial cells. (*Source*: Wells and

Isolation and purification of nucleic acids

- A prerequisite for in vitro gene technology is to prepare large quantities of relatively pure nucleic acids from the desired organism.
- After disruption of the cells the nucleic acids must be separated from other cellular components using a variety of techniques including centrifugation, electrophoresis, adsorption and various forms of precipitation.
- *Cutting DNA molecules.* DNA can be cut using mechanical or enzymatic methods. The non-specific mechanical shearing will generate random DNA fragments, which are most often used to create genomic libraries.
- This crude method does not permit the isolation of a specific fragment containing a known gene or operon.
- In contrast, when specific restriction endonuclease enzymes are used it is possible to recognise and cleave specific target base sequences in double-stranded (ds) DNA.

- Restriction endonucleases are able to sever the phosphodiester backbone of both strands of the DNA to generate $3'OH$ and $5'PO_4$ termini .
- Large numbers of different restriction endonucleases have been extracted and classified from a wide variety of microbial species. Restriction endonucleases are named according to the species from which they were first isolated, e.g. enzymes isolated from *Haemophilus influenzae* strain Rd are designated *Hind* and when several different restriction enzymes are isolated from the same organism they are designated *HindI*, *HindII* etc.
- Restriction endonucleases can distinguish between DNA from their own cells and foreign DNA by recognising a certain sequence of nucleotides.
- This allows the breaking open of a length of DNA into shorter fragments that contain a number of genes determined by the enzyme used. Such DNA fragments can then be separated from each other on the basis of different molecular weight.
- *Splicing DNA*. DNA fragments with either blunt ends or with cohesive overlapping ends can be joined together in vitro by the action of specific DNA ligases.
- The DNA ligase that is widely used was encoded by phage T4.
- *Eco*RV cuts symmetrically leaving blunt ends $3'OH$ groups at the terminus of one strand with the $5'PO_4$ terminus of another strand provided that the ends are complementary.

- The sources of 'passenger' can be quite different, giving an opportunity to replicate the DNA biologically by inserting it into other cells.

- The composite molecules in which DNA has been inserted have also been termed 'DNA chimaeras' because of the analogy with the Chimaera of mythology, a creature with the head of a lion, the body of a goat and the tail of a serpent.

- *The vector or carrier system.* Two broad categories of expression vector molecules have been developed as vehicles for gene transfer, *plasmids* (small units of DNA distinct from chromosomes) and *bacteriophages* (or bacterial viruses).

- Vector molecules will normally exist within a cell in an independent or extrachromosomal form not becoming part of the chromosomal system of the organism. Vector molecules should be capable of entering the host cell and replicating within it.

- Ideally, the vector should be small, easily prepared and must contain at least one site where integration of foreign DNA will not destroy an essential function.

- Plasmids will undoubtedly offer the greatest potential in biotechnology and have been found in an increasingly wide range of organisms, for example, bacteria, yeasts and mould fungi; they have been mostly studied in Gram-negative bacteria.

- Expression vectors can often add an affinity tag to the protein to facilitate its purification by affinity chromatography. The tag can later be removed.

- *Introduction of vector DNA recombinants*. The new recombinant DNA can now be introduced into the host cell by transformations (the direct uptake of DNA by a cell from its environment) or transductions (DNA transferred from one organism to another by way of a carrier or vector system) and if acceptable the new DNA will be cloned with the propagation of the host cell.

Table 3.1 **Strategies involved in genetic engineering**

Formation of DNA fragments	Extracted DNA can be cut into small sequences by specific enzymes, restriction endonucleases, found in many species of bacteria.
Splicing of DNA into vectors	The small sequences of DNA can be joined or spliced into the vector DNA molecules by an enzyme DNA ligase creating an artificial DNA molecule.
Introduction of vectors into host cells	The vectors are either viruses or plasmids, and are replicons and can exist in an extrachromosomal state; transfer normally by transduction or transformation.
Selection of newly acquired DNA	Selection and ultimate characterisation of the recombinant clone.

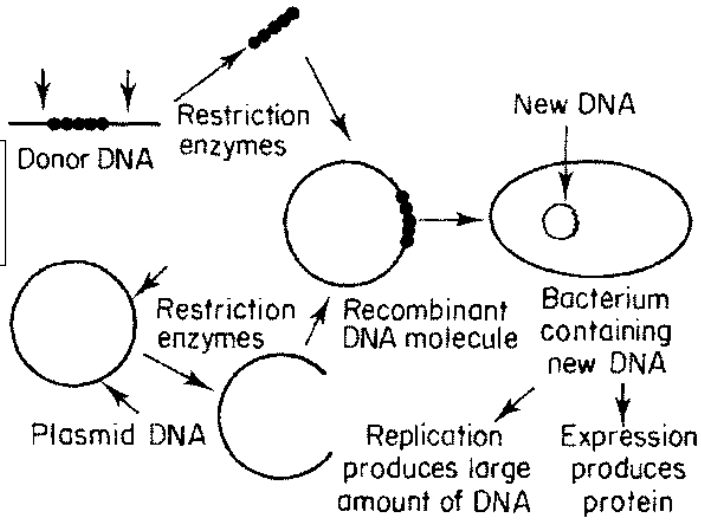


Fig. 3.6 Recombinant DNA: the technique of recombining genes from one species with those of another.

- Novel methods of ensuring DNA uptake into cells include *electro-poration* and *mechanical particle delivery* or *biolistics*.
- Electroporation is a process of creating transient pores in the cell membrane by application of a pulsed electric field. Creation of such pores in a membrane allows introduction of foreign molecules, such as DNA,
- RNA, antibodies, drugs, etc., into the cell cytoplasm. Development of this technology has arisen from synergy of biophysics, bioengineering and cell and molecular biology.
- While the technique is now widely used to create transgenic microorganisms, plants and animals, it is also being increasingly

used for application of therapeutics and gene therapy.

- The mechanical particle delivery or 'gene gun' methods deliver DNA on microscopic particles into target tissue or cells.
- This process is increasingly used to introduce new genes into a range of bacterial, fungal, plant and mammalian species and has become a main method of choice for genetic engineering of many plant species including rice, corn, wheat, cotton and soybean.

7. What is phylogenetic tree? Give the step involved in building phylogenetic tree with a sequence. (May 2016)

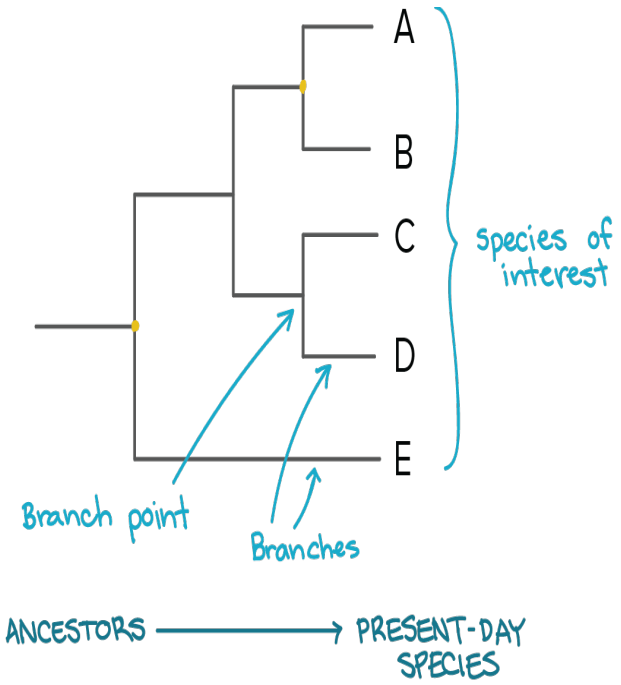
- A **phylogenetic tree** is a diagram that represents evolutionary relationships among organisms. Phylogenetic trees are hypotheses, not definitive facts.
- The pattern of branching in a phylogenetic tree reflects how species or other groups evolved from a series of common ancestors.
- In trees, two species are **more related** if they have a more recent common ancestor and **less related** if they have a less recent common ancestor.
- Phylogenetic trees can be drawn in various equivalent styles. Rotating a tree about its branch points doesn't change the information it carries.
- Humans as a group are big on organizing things. Not necessarily things like closets or rooms; I personally score low on the organization front for both of those things. Instead, people often like to group and order the things they see in the world around them.
- Starting with the Greek philosopher Aristotle, this desire to classify has extended to the many and diverse living things of Earth.
- Most modern systems of classification are based on evolutionary

relationships among organisms – that is, on the organisms' **phylogeny**. Classification systems based on phylogeny organize species or other groups in ways that reflect our understanding of how they evolved from their common ancestors.

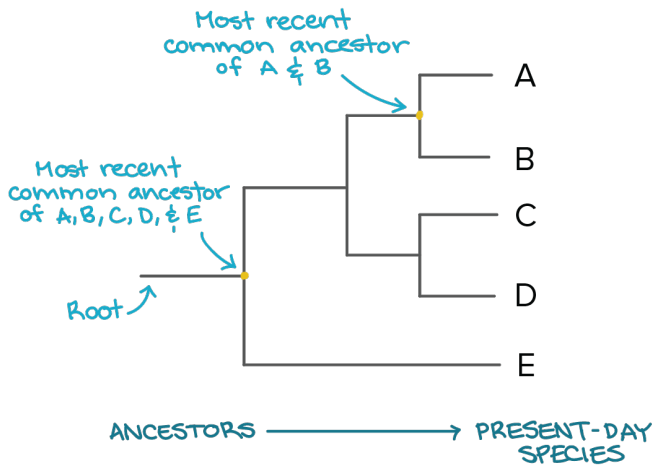
- In this article, we'll take a look at **phylogenetic trees**, diagrams that represent evolutionary relationships among organisms. We'll see exactly what we can (and can't!) infer from a phylogenetic tree, as well as what it means for organisms to be more or less related in the context of these trees.

Anatomy of a phylogenetic tree

- When we draw a phylogenetic tree, we are representing our best hypothesis about how a set of species (or other groups) evolved from a common ancestor^11start superscript, 1, end superscript.
- As we'll explore further in the article on building trees, this hypothesis is based on information we've collected about our set of species – things like their physical features and the DNA sequences of their genes
- In a phylogenetic tree, the species or groups of interest are found at the tips of lines referred to as the tree's **branches**. For example, the phylogenetic tree below represents relationships between five species, A, B, C, D, and E, which are positioned at the ends of the branches:



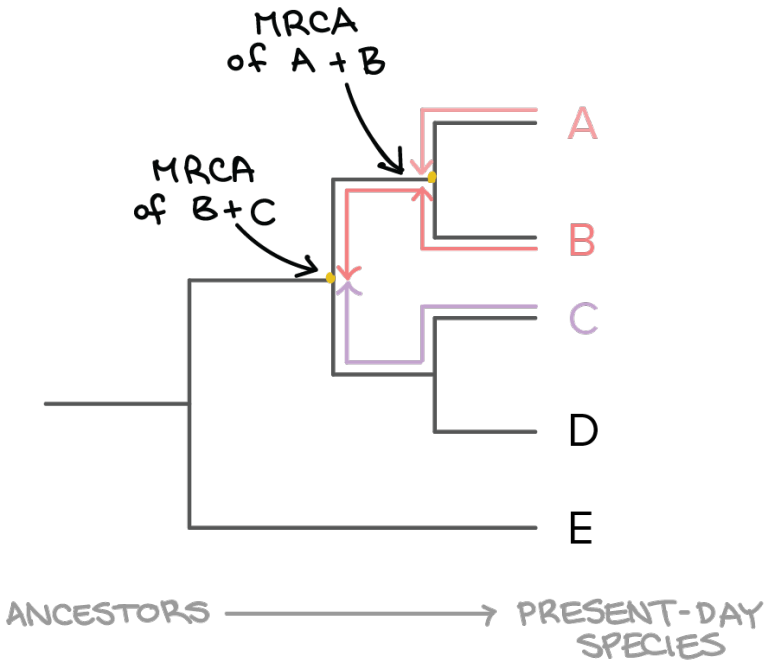
- The pattern in which the branches connect represents our understanding of how the species in the tree evolved from a series of common ancestors.
- Each branch point (also called an **internal node**) represents a **divergence** event, or splitting apart of a single group into two descendant groups.
- At each branch point lies the **most recent common ancestor** of all the groups descended from that branch point. For instance, at the branch point giving rise to species A and B, we would find the most recent common ancestor of those two species. At the branch point right above the **root** of the tree, we would find the most recent common ancestor of all the species in the tree (A, B, C, D,E)



Taxonomy and phylogeny:

- Each horizontal line in our tree represents a series of ancestors, leading up to the species at its end. For instance, the line leading up to species E represents the species' ancestors since it diverged from the other species in the tree.
- Similarly, the root represents a series of ancestors leading up to the most recent common ancestor of all the species.
    - In a phylogenetic tree, the **relatedness** of two species has a very specific meaning. Two species are *more* related if they have a *more recent* common ancestor, and *less* related if they have a *less recent* common ancestor.
- We can use a pretty straightforward method to find the most recent common ancestor of any pair or group of species. In this method, we start at the branch ends carrying the two species of interest and “walk backwards” in the tree until we find the point where the species’ lines converge.
- For instance, suppose that we wanted to say whether A and B or B and C are more closely related. To do so, we would follow the lines of both pairs of species backward in the tree. Since A and B converge at a common ancestor first as we move backwards, and B only converges with C after its junction point with A, we

can say that A and B are more related than B and C.

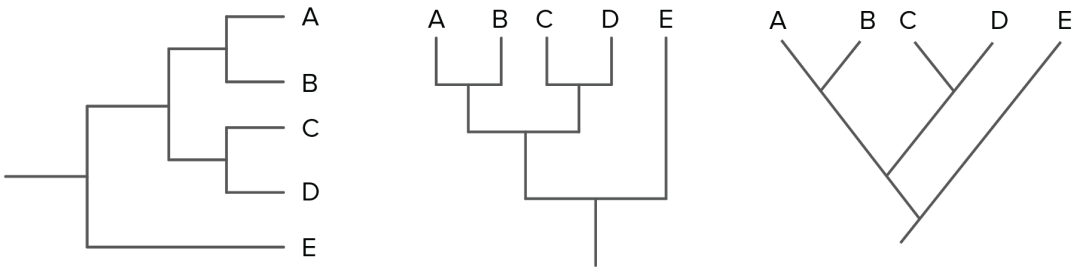


Taxonomy and phylogeny:

- Importantly, there are some species whose relatedness we can't compare using this method.
- For instance, we can't say whether A and B are more closely related than C and D. That's because, by default, the horizontal axis of the tree doesn't represent time in a direct way.
- So, we can only compare the timing of branching events that occur on the same lineage (same direct line from the root of the tree), and not those that occur on different lineages.

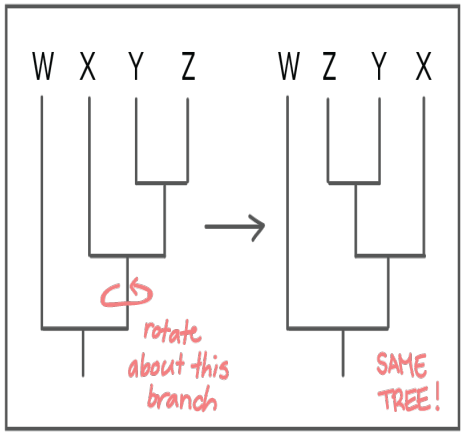
Some tips for reading phylogenetic trees

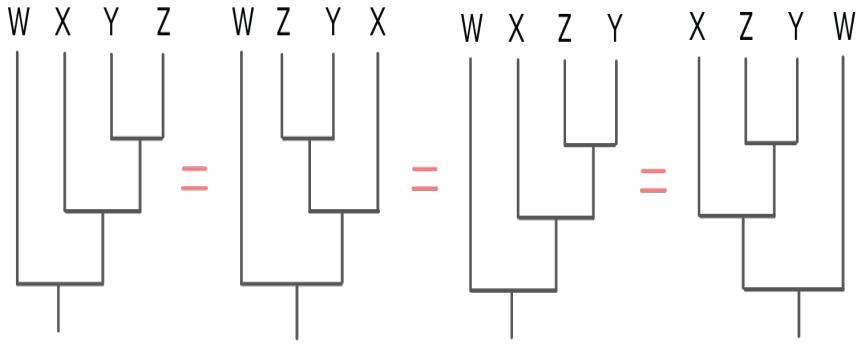
You may see phylogenetic trees drawn in many different formats. Some are blocky, like the tree at left below. Others use diagonal lines, like the tree at right below. You may also see trees of either kind oriented vertically or flipped on their sides, as shown for the blocky tree.



Taxonomy and phylogeny: Figure 2

- The three trees above represent identical relationships among species A, B, C, D, and E. You may want to take a moment to convince yourself that this is really the case – that is, that no branching patterns or recent-ness of common ancestors are different between the two trees.
- The identical information in these different-looking trees reminds us that it's the branching pattern (and not the lengths of branches) that's meaningful in a typical tree.
- Another critical point about these trees is that if you rotate the structures, using one of the branch points as a pivot, you don't change the relationships. So just like the two trees above, which show the same relationships even though they are formatted differently, all of the trees below show the same relationships among four species:





Taxonomy and phylogeny: Figure 3

- If you don't see right away how that is true (and I didn't, on first read!), just concentrate on the relationships and the branch points rather than on the ordering of species (W, X, Y, and Z) across the tops of the diagrams.
- That ordering actually doesn't give us useful information. Instead, it's the branch structure of each diagram that tells us what we need to understand the tree.
- So far, all the trees we've looked at have had nice, clean branching patterns, with just two lineages (lines of descent) emerging from each

branch point. However, you may see trees with a **polytomy** meaning a branch point that has three or more different species coming off of it^22squared.

ELEMENTS OF BIOTECHNOLOGY – BM E52

UNIT-5

2 MARK:

1.Define biopolymers. Give examples. (NOV 2014, NOV 2015, MAY 2016, NOV 2017,NOV 2018)

- Biopolymers are organic molecules that are composed by repeating monomers and produced by living organisms.
- Biopolymers are a diverse and remarkably versatile class of materials hat either produced by biological systems or synthesized from biological source materials.
- New biocompatible an biodegradable biopolymers are produced from plants, microbes, animals, renewable agricultural wastes, and feedstocks.

2. Write a note on Ti plasmids.(NOV 2014)

- Ti plasmids are plasmids from the genus Agrobacterium that encode a natural system of plant transformation. They are used extensively as vectors for plant genetic engineering.
- The Ti plasmid carries the genes for opine synthesis by plant cells as well as the corresponding catabolism genes.
- All Ti plasmids code for functions associated with i) plasmid replication and .sensory perception of exogenous signals released by the plant host and neighboring agrobacterial cells at the site of infection .

3. Write down the importance of biotechnology in food industry.(NOV 2015)

- Biotechnology is used in the production of food constituents; flavours, aroma, food additives and an array of other high valued-enhanced products, genetically modified organisms and crops.

- Food testing and in diagonostics of food ingredients the utilization of advanced technologies of biotechnology is done.
- Biotechnology has a long history of use in food production and processing. ... Selective breeding of essential foods such as rice, corn and wheat have created thousands of local varieties with improved yield compared to their wild ancestors.

4.What are transgenic crops? (APRIL/MAY 2016)

- A transgenic crop is a genetically modified organism (GMO).
- Transgenic crops are crops into which one or more genes from another species have been introduced into the genome, using genetic engineering processes.

5.Write a note on plasmid. (NOV 2016)

- A plasmid is a small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently.
- Plasmids will undoubtedly offer the greatest potential in biotechnology and have been found in an increasingly wide range of organisms.

6.Define Fermentation. (NOV 2016,MAY 2019)

- Bioprocess or fermentation technology normally involve complete living cells,organelles or enzymes as the biocatalyst.
- Fermentation were derived in part from the use of microorganisms for the production of foods such as cheeses, yoghurts, pickles and sausages, soya sauce and other Oriental products, and beverages

7.List the application of Biotechnology in Agriculture. (MAY 2017)

- rDNA technology
- Tissue culture

- somatic hybridisation
- embryo rescue
- molecular diagnostics

8.What is meant by Bio-pesticides?Give an example with its mode of action (MAY 2017,NOV/DEC 2017,APRIL/MAY 2018,NOV/DEC 2018,MAY 2019)

- The microorganisms applied in the field to control pests and disease is called as Bio-pesticides.
- Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals.

Mode of action:

1. Microbial bioinsecticides

2. Plant-incorporated protectants

3. Bioinsecticides

9.Give example of Hot and cold beverage. (APRIL/MAY 2018)

Hot beverage example:

1. Apple cider
2. Hot mulled wine
3. Coffee
4. Hot chocolate
5. Tea

Cold beverage example:

1. Fruit Juice
2. Ice Tea, ice coffee
3. Punch
4. Beer
5. Lemonade

10.What is a Bioreactor? (NOV/DEC 2018)

A main function of a bioreactor is to minimise the cost of producing a product or service. Bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms.

11.Give some uses of plant cell culture.(NOV 2020)

- Plant tissue culture is used widely in the plant sciences, forestry, and in horticulture.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- Large scale production of artificial seeds through somatic embryogenesis.

12.What is the impact of Biotechnology in food and beverage industry? (NOV 2020)

- Increase the crops yield through introducing high-yielding varieties resistant to biotic and abiotic stresses
- Increase the nutritional values of foods which is a very important factor in rural areas.
- Improvement of functional beverages, flavor enhancement, bio-preservation and enzyme modification.

11- Marks

1.Write a note on beverage. Explain in detail about beer production.(NOV 2014)

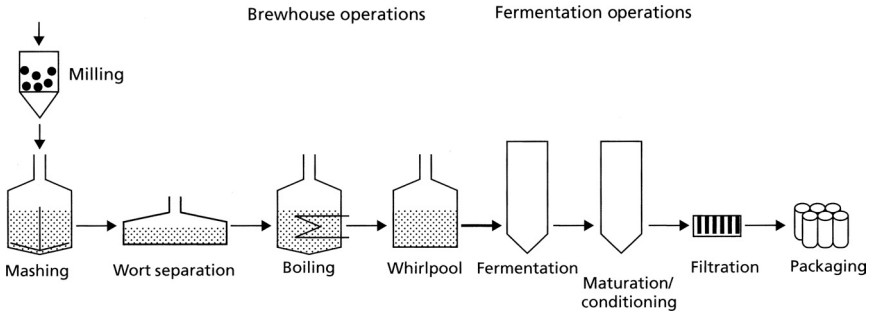
- Fermented foods and beverages have a significant role in all societies, and result from the action of microorganisms or enzymes on a wide range of agricultural materials with associated desirable biochemical changes giving significant organoleptic improvements to the final product.
- As a result of the fermentation process the product is usually more nutritious, more digestible, has improved flavour and is toxicologically

and microbiologically safer.

- Fermented foods and beverages derived from plant and animal materials are an accepted and essential part of the diet in almost all parts of the world, involving a wide diversity of raw materials as substrates, using technology from the most primitive to the most advanced, and achieving an astounding range of sensory and textural qualities in the final products. Fermented foods include breads, cheeses, yoghurts, sauerkraut, soy sauce, tempeh, mushrooms, etc., while fermented beverages include alcoholic beers, wines, sake, brandy, whisky, and non-alcoholic tea, coffee and cocoa.

BEER PRODUCTION

- The earliest record of brewing was inscribed in cuneiform characters on clay tablets in Sumaria (present day Iraq) at least 6000 years ago.
- How- ever, it is quite possible that primitive forms of brewing existed many thousands of years earlier. Beer can be defined as 'a drink obtained by the alcoholic fermentation of an aqueous extract of germinated cereal with addition of hops'.
- Beer is a relatively poor medium for bacterial growth, largely due to its low pH, content of antiseptics such as carbon dioxide, alcohol, hop extracts and its low temperature of storage. Pathogens cannot live in beer thus making it safer to drink than water in many countries. Because of its complex biochemical content, beer is almost impossible to analyse.
- Beers, ales and lagers are produced mostly from starchy cereals such as barley. Additional carbohydrate sources, known as adjuncts, are normally added in varying proportions. In practice, there are five major steps in the manufacture of beers from grains: malting, mashing, fermentation, maturation and finishing.



MALTING

Dried barley is soaked or steeped in water and then spread out on the malthouse floor or in revolving drums, where the seeds germinate with the formation of starch-degrading (amylase) and protein-degrading (protease) enzymes. The germinated seeds are then killed by kilning (slow heating to 80°C) while still retaining most of the enzyme activity (malt).

MASHING

In this stage the malt is mixed with hot water (55–65°C), and the starches and proteins break down to produce dextrins, maltose and other sugars, protein breakdown products, minerals and other growth factors (the wort). This is the medium for the beer fermentation. Hops may be added prior to the fermentation to give characteristic flavour and some antiseptic properties.

FERMENTATION

- The wort is transferred to open bioreactor systems and inoculated with pure strains of yeast. In Britain a top-fermentin Saccharomyces cerevisiae is used at 20–28°C to produce beers, ales or stouts.
- In continental Europe bottom-fermenting yeast Saccharomyces uvarum ferments the wort at a lower temperature (10–15°C) to produce lager. Light or low-alcohol beers are usually derived from lagers, which contain fewer fermentable substrates and consequently

less alcohol production and fewer calories. Such styles of beers have become very popular in North America and the UK.

- The fermentation of glucose is anaerobic and can be summarised by the following equation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Glucose ethanol carbon dioxide

Theoretical yield

180 g 92 g 88 g

Maturation and finishing

- Beer is usually matured in casks at 0°C for several weeks to improve flavour, settle out the y easts and remove haze. Bottled or canned beers are usually pasteurised at 60–61°C for 20 minutes. The alcohol content of beer is usually between 4 and 9%; with ales it is somewhat higher.

- While European type lagers and beers are now produced worldwide, the traditional beer in India and Asia is rice beer and in Africa, sorghum beer. Sorghum beer is a very crude material rich in solids and vitamins and is, in fact, a valuable source of nutrition to those who drink it. Approximately 1.2 billion hectolitres (1 hl = 100 l) of beer are consumed annually worldwide. Without doubt, beer is the most consumed alcoholic beverage.

- Traditional applied genetics, together with protoplast fusion and technology, are constantly improving the yeast strains used in these fermentations. In particular, there has been a vast upsurge in genetic engineering knowledge of Saccharomyces cerevisiae. A new commercial brewing yeast has been developed and approved using recombinant DNA techniques.

2. Discuss the role of biotechnology in agricultural field.(MAY 2016,NOV 2017,NOV 2018, MAY 2019)

Agriculture

- Genetically modified crops ("GM crops", or "biotech crops") are plants used in agriculture, the DNA of which has been modified with genetic engineering techniques. In most cases, the main aim is to introduce a new trait that does not occur naturally in the species. Biotechnology firms can contribute to future food security by improving the nutrition and viability of urban agriculture. Furthermore, the protection of intellectual property rights encourages private sector investment in agro biotechnology. For example, in Illinois FARM
- Illinois (Food and Agriculture RoadMap for Illinois) is an initiative to develop and coordinate farmers, industry, research institutions, government, and nonprofits in pursuit of food and agriculture innovation. In addition, the Illinois Biotechnology Industry Organization (iBIO) is a life sciences industry association with more than 500 life sciences companies, universities, academic institutions, service providers and others as members. The association describes its members as "dedicated to making Illinois and the surrounding Midwest one of the world's top life sciences centers."
- Examples in food crops include resistance to certain pests, diseases, stressful environmental conditions, resistance to chemical treatments (e.g. resistance to a herbicide), reduction of spoilage, or improving the nutrient profile of the crop. Examples in non-food crops include production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation.

Genetically Modified Organisms

You must have heard the term 'GMO' used by people around you or in the news every

now and then. What does this mean? GMO stands for 'Genetically Modified Organisms'. GMOs are plants, animals, bacteria or fungi whose genes have been modified by genetic manipulation. Genetically modified crops or GM crops are used in the following ways:

- They are more tolerant to stresses such as drought, cold, heat etc.
- They are pest-resistant and therefore less dependent on chemical pesticides.
- Genetically Modified crops help to reduce post-harvest losses.
- They help to increase the mineral usage by plants, thereby preventing early exhaustion of soil fertility.
- Genetically modified crops have enhanced nutritional value. Example – Vitamin A enriched rice.

Genetic modifications also help to create tailor-made plants to provide alternative resources to industries, such as fuels, starches, and pharmaceuticals. Let's look at some examples of GM crops and how they are useful.

Bt Cotton

- This is a genetically modified version of cotton. 'Bt' stands for the microbe Bacillus thuringiensis. This microbe produces an insecticidal protein or toxin that kills other insects such as tobacco budworm, flies, mosquitoes, beetles etc. This is because it stays inactive (as protoxin) in the Bacillus. It gets activated only once it comes in contact with the alkaline pH in the insect gut when the insect ingests it. The activated toxin then binds to the surface of epithelial cells and creates pores in it. This causes the cells to swell and lyse, eventually leading to the death of the insect.

- Scientists isolated the Bt toxin genes from Bacillus thuringiensis and incorporated it into various crop plants such as cotton. This variety is 'Bt cotton'.

Since most Bt toxins are insect-group specific, the choice of genes to be incorporated depends on the crop and the targeted pest. A gene named cry codes for the toxin protein and there a number of these genes. For example, the genes cryIAc and cryIIAb encode toxins that control cotton bollworms whereas the gene cryIAb controls the insect 'corn borer'.

Pest Resistant Plants

- Several nematodes live as parasites on multiple hosts like plants, animals, and even human beings. A specific nematode 'Meloidegyne incognitia' infects the roots of tobacco plants and causes a great decrease in yield. To prevent this infestation, a novel strategy was adopted which is based on the process of RNA interference (RNAi).

- RNAi is a method of cellular defence in all eukaryotes. It involves the silencing of a specific mRNA by a complementary double-stranded (ds) RNA that binds and inhibits the translation of this mRNA. The complementary RNA can come from an infection by viruses that have RNA genomes or genetic elements called 'transposons'.

- Scientists took advantage of this process and introduced nematode-specific genes into host plants using Agrobacterium vectors. The introduced DNA produces both sense and anti-sense strands in the host cells. These complementary strands then produce dsRNA and initiate RNAi and thus silence the specific RNA of the nematode. Consequently, the parasite cannot survive in the host that expresses this RNA, leading to resistance against that parasite.

Improved post-harvest characteristics

- Losses during storage and transport of some crops can be as high as 40% in the USA and Europe, and as high as 80% elsewhere. While a great deal of this loss will be due to diseases and pests, with soft fruits and vegetables there can be bruising, heat and cold damage,

over-ripeness, off flavours and odours, etc.

- Genetic manipulation of ornamental plants and floriculture involving flower and leaf colour, abundance of flavours, perfume and shape are now major targets for the decorative plant industries, especially in Germany, Australia and the USA.

Improved resistance to specific herbicides

- The killing of plant weed species by the application of selective herbicides gives a growth advantage to commercial crop plants. Such compounds have been designed to disrupt the growth of certain weed species without affecting the particular crop plant. The annual global herbicide market is in excess of US$6 billion. However, there is increasing opposition to the continued use of such chemical compounds from environmental and human health considerations. Herbicide-tolerant crop plants have now been produced by genetically engineering genes resistant to specific herbicides. This has been seen as a way of producing more effective, less costly and more environmentally compatible weed control.

- Green biotechnology is biotechnology applied to agricultural processes. An example would be the selection and domestication of plants via micropropagation. Another example is the designing of transgenic plants to grow under specific environments in the presence (or absence) of chemicals.

- One hope is that green biotechnology might produce more environmentally friendly solutions than traditional industrial agriculture. An example of this is the engineering of a plant to express a pesticide, thereby ending the need of external application of pesticides.

- An example of this would be Bt corn. Whether or not green biotechnology products such as this are ultimately more environmentally friendly is a topic of considerable debate.

3.Comments on food and beverage industries. (NOV 2016, MAY 2017, NOV 2017, MAY 2018, MAY 2019)

- Food production is the largest worldwide industry, and in industrialised nations the expenditure on food can account for at least 20–30% of household budgets. However, whereas food is in general in excessive pro- duction in most parts of the world, scarcity and insufficient production exists in Africa, Central China and most parts of South America. The food industry has evolved through specialist trades or occupations, e.g. butchers, bakers, confectioners, etc., to national and multinational organisations involved in the manufacture and distribution of food on a worldwide scale. With the improvement in means of transportation, foods are available on a worldwide basis, and developments in food preservation methods give independence from seasonal availability.
- In essence, the food industry now serves the function of supplying society with high-quality, wholesome foods, all the year round, and at a distance, in time and location, from the place of primary production.

Fermented food and beverages.

- Fermented foods and beverages have a significant role in all societies, and result from the action of microorganisms or enzymes on a wide range of agricultural materials with associated desirable biochemical changes giving significant organoleptic improvements to the final product. As a result of the fermentation process the product is usually more nutritious, more digestible, has improved flavour and is toxicologically and microbiologically safer.

- Fermented foods and beverages derived from plant and animal materials are an accepted and essential part of the diet in almost all parts of the world, involving a wide diversity of raw materials as substrates, using technology from the most primitive to the most advanced, and achieving an astounding range of sensory and

textural qualities in the final products. Fermented foods include breads, cheeses, yoghurts, sauerkraut, soy sauce, tempeh, mushrooms, etc., while fermented beverages include alcoholic beers, wines, sake, brandy, whisky, and non-alcoholic tea, coffee and cocoa.

Alcoholic beverages

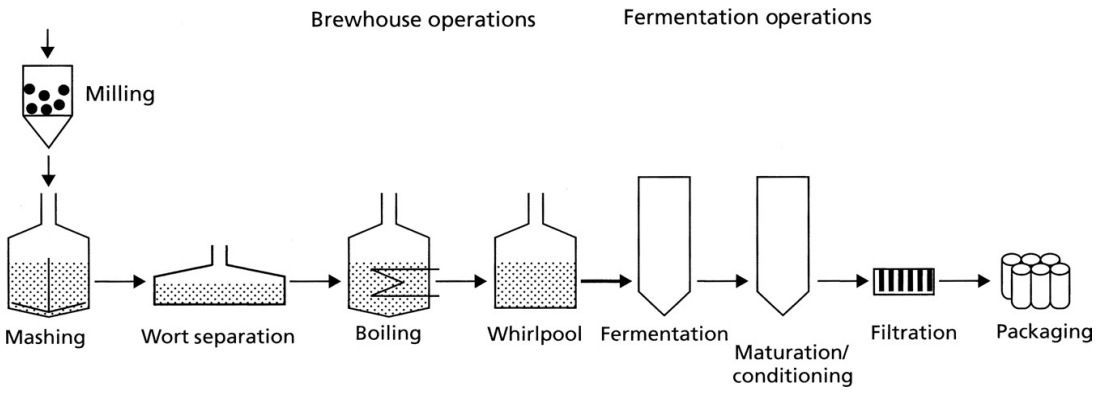
- Alcoholic beverages occur throughout the world in many different forms and tastes. The types of beverage produced in any particular region or country almost entirely reflect the crops grown.
- The starting material normally comprises either sugary materials (fruit juices, plant sap, honey) or starchy materials (grains or roots), which need to be hydrolysed to simple sugars before the fermentation .When these substrates are incubated with suitable microorganisms and allowed to ferment, the end-product is a liquid containing anything from a few per cent up to 16% or more of alcohol, with an acid pH and depleted in nutrients for most contaminating microorganisms; these factors combine to give the product a certain degree of biological stability and safety.

Wines

- Most commercial wines use the wine grape Vitis vinifera, and cultivars of this species have been transported throughout the world to establish new wine-producing areas. Soil quality can have an important and subtle effect on the eventual quality of the wine. Red wine is formed when black grapes are crushed and fermented whole. In contrast, if the skins are removed from black grapes or when white grapes are used, white wine is the final product. Hundreds of different wines are recognised in the many producing areas of the world. Ros´e wine results from some limited contact with the skins of black grapes, dry wine is the end-product of complete sugar utilisation while sweet wine will still retain some residual sugars.
- Harvesting time of the grapes is judged largely by artisan skills, and the grapes, containing 15–25% sugar, are then crushed mechanically or by treading of feet. The juice (now termed must) is the substrate for the truly

biotechnological stage of the production. Since the must will contain many contaminating yeasts and bacteria it is usual practice to add SO2 to control or abolish this natural fermentation capacity. In large-scale wine produc- tion the

- must is partially or completely sterilised, inoculated with the desired strain of yeast, Saccharomyces cerevisiae var. ellipsoideus, and subjected to controlled fermentation in suitable tanks or bioreactors. The dryness or sweetness of the wine will depend on the degree of sugar conversion, glycerol levels, secondary infections, etc.
- Fermentation conditions such as time and temperature will depend on the type of wine desired. After fermentation, the wines are run into storage vats or tankers where the temperature quickly drops, precipitates form and subtle chemical changes take place. Many wines undergo a spontaneous secondary bacterial (Leuconostoc spp.) or malolactic fermentation, converting residual malic acid to lactic acid. The final alcoholic content of wines ranges between 10 and 16%.
- Modern scientific research now supports the view that moderate wine consumption is associated with lower coronary heart disease mortality. As Louis Pasteur stated 'Wine is the most healthful and most hygienic of beverages'.
- Fortified wines, such as sherry, port and vermouth, are wines to which additional alcohol is added after fermentation, raising the alcohol level to about 20%.



4.Write the applications of biotechnology in food industry.(NOV 2015, NOV 2017)

Coffee, tea and cocoa

- In Asia, India, Africa and South America, non-alcoholic fermented beverages are derived from coffee, tea and cocoa plants. These beverages have gained worldwide approval and high commercial value.
- Tea is derived from the enzymic action released after the crushing of the leaves, while for coffee and cocoa, the pulp surrounding the beans is removed in part by a natural fermentation with bacteria, yeast and fungi, which is critically important for full flavour and aroma development. The dried products, tea leaves, and coffee and cocoa beans, can then be shipped throughout the world and the final beverage formed by the addition of water.
- Little is known about the exact microbial contribution to these fermentation processes. The processes are still empirical with little exact science. Huge quantities of these products are consumed worldwide and form the economic basis of several multinational companies.

Dairy products

- The manufacture of cultured dairy products represents the second most important fermentation (after the production of alcoholic beverages), accounting for over 20% of fermented foods/drinks produced worldwide.
- The origin of the development of dairy products, such as fermented milk, butter and cheeses, is lost in antiquity. Such fermentations are related to areas with high numbers of lactating animals, cows, goats and sheep, and Europe is the major world area of production.
- Worldwide, fermented dairy products account for about 10% of all fermented food production. It is now known that these

fermentations result largely from the activity of a group of bacteria called lactic acid bacteria.

- ❖ Fermentation by lactic acid bacteria results in preservation and transformation of milk, and has been used unknowingly for thousands of years. In the past, these fermentations arose directly from the natural occurrence of lactic acid bacteria, but gradually it was recognised that a portion of a previously successful 'ferment' when added to milk gave better results.
- ❖ Nowadays, an inoculum (a pure starter culture) of selected bacteria is generally added to the milk to be fermented. The modern worldwide dairy industries owe much to the development of pure starter cultures, good fermentation practices and strict adherence to hygienic protocol. In the USA alone, these bacteria are involved in the manufacture of food products with an annual value of US$20–30 billion.
- ❖ The lactic acid bacteria can have many beneficial effects in the foods in which they grow.

(1) They have an inhibitory effect (bacteriocins) on many undesirable bacteria while they themselves are generally harmless; in this way they preserve the milk.

(2) They produce highly acceptable texture and flavour modifications in the milk.

(3) Reputedly, they have beneficial health effects on intestinal microflora (probiotics).

- When growing in milk, these beneficial bacteria break down lactose to lactic acid. However, many other reactions can occur, depending on the composition of the substrate, types of additives and mode of fermentation. These can result in many other metabolites being formed, giving distinctive flavour and appearance to the milk products, e.g.

buttermilk, sour cream, yoghurt and the vast range of cheeses.

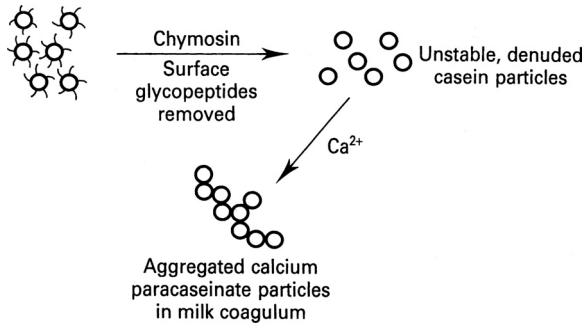
Cheese production from milk is essentially a dehydration process in which the milk protein (casein) and fat are concentrated between 6 and 12 times. The common, basic steps in most cheese productions are:

(1) acidification of the milk by the conversion of the sugar lactose into lactic acid by the lactic acid bacteria

(2) coagulation of the casein by a combination of proteolysis and acidification.

Proteolysis is started by the rennet (chymosin enzyme) (animal or fungal origin) and the coagulated caseins form a gel, which entraps any fat present .

The separated curd is cut into blocks, drained and pressed into shapes, matured and made into cheeses. The details of cheese production are very complicated and involve many individual strains of bacteria and in some cases filamentous fungi (camembert, blue cheese), special milks, selected additives and differing process techniques, which cannot be covered here.



However, an important recent biotechnological innovation in cheese production has been the use of recombinant DNA techniques for chymosin production and commercial use. At present there are six sources of commercial rennet: three from animals (veal calves, adult

cows and pigs); and three fungal sources. The fungal sources are almost identical in function to the animal chymosins and account for approxi- mately one-third of world cheese production – particularly in the USA and France. However, they can on occasion cause yield reductions and poor flavour when compared to animal chymosins.

The second major group of dairy products are the yoghurts. They are major foods consumed worldwide and represent one of the fastest growing food products in the food industry. Claims are now being made that live yoghurt bacteria can exist transitorily in the human gut with benefits to the digestive and other systems.

Traditionally, yoghurt is fermented whole milk; the process uses a mixed culture of Lactobacillus bulgaricus and Streptococcus thermophilus. The characteristic flavour compound, acetaldehyde, is produced by Lb. bulgaricus

Unripened cheeses

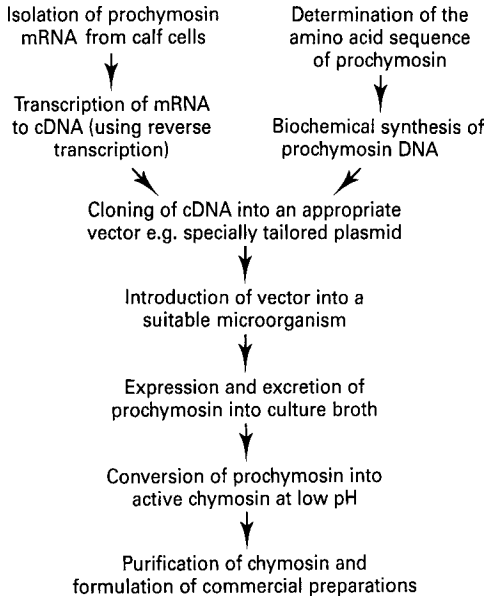
Low fat (cottage cheese) High fat(cream cheese)

Ripened cheeses

Hard cheese (internal ripening)

Ripened by bacteria (Cheddar and Swiss cheese) Ripened by mould (Roquefort and other blue cheeses)

Soft cheeses (ripening proceeds from outside) Ripened by bacteria (Limburger)



while the St. thermophilus generates the fresh acid taste by the conversion of lactose to lactic acid. Both bacteria produce extracellular polymers that give the characteristic viscosity of the product. Incubation is at 30 or 45°C. Set yoghurt is packed into the container after inoculation and allowed to ferment in the container. Frozen yoghurt is gaining increasing popularity as an alternative for ice cream.

5. What are food products derived from microbes cells.(MAY 2016, MAY 2017, MAY 2018)

In the 1950s, agriculturalists and nutritionists were becoming concerned that in the near future traditional sources of protein, such as cattle, pigs and poultry, would not be adequate for the increasing world demand. In essence, they envisaged a global shortage of protein foods resulting in extensive protein malnutrition, as was being seen in some developing countries with the childhood diseases of kwashiorkor and marasmus.

The time required to double the mass of various organisms

Organism	Time required to double biomass
Bacteria and yeasts	20–120 minutes
Moulds and algae	2–6 hours
Grass and some plants	1–2 weeks
Chickens	2–4 weeks
Pigs	4–6 weeks
Cattle (young)	1–2 weeks
Humans (young)	3–6 months

Microorganisms produce protein much more efficiently than any farm animals . The protein-producing capacities of a 250 kg cow and 250 g of microorganisms are often compared. Whereas the cow will put on 200 g of protein a day, the microbes, in theory, could produce 25 tonnes in the same time under ideal growing conditions. However, the cow also has the unique ability to convert grass into protein-rich milk. No rival method has ever been developed. The cow has been described as 'a live, self-reproducing and edible bioreactor'. The challenge was then set to pro- duce economically large quantities of protein-rich microbial biomass utilis- ing modern fermentation/bioreactor technology. The potential advantages of using microorganisms for protein production.

SCP derived from high-energy sources

Prior to the dramatic escalation of oil prices by the oil-producing nations in the 1970s, there was a wide range of SCP fermentation processes being developed utilising gas-oil, methanol, ethanol,

methane or n-alkanes. Even at that time the wisdom of using high energy-potential compounds for food production was being questioned by many scientists. How right they were! Most oil and gas companies had large gluts of petroleum by-products and consequently had large research and production facilities devoted to SCP production.

The potential advantages of using microbes for SCP production

- Microorganisms can grow at remarkably rapid rates under optimum conditions; some microbes can double their biomass every 0.5–1.0 hours.

· Microorganisms are more easily modified genetically than plants and animals; they are more amenable to large-scale screening programmes to select for higher growth rate, improved amino acid content, etc., and can be more easily subjected to gene transfer technology.

· Microorganisms have relatively high protein content and thenutritional value of the protein is good.

· Microorganisms can be grown in vast numbers in relatively small, continuous-fermentation processes, using relatively small land area and are also independent of climate.

· Microorganisms can grow on a wide range of raw materials, inparticular low-value wastes, and some can also use plant-derivelignocellulose.se

SCP from waste organic materials

The materials that make up waste organics should normally be recycled back into the ecosystem, e.g. carbohydrate wastes, sugars, starch, whey, molasses; lignocellulosic waste, straw, bagass, oil- and date-palm, as well as animal manures. It was proposed that many of these wastes could be trans- formed into edible protein for animals and, possibly, human consumption. Over the years, there have been many feasibility studies carried out world- wide, but only two approaches have achieved acceptance as worthwhile contributions to human protein consumption: Quorn™ myco-protein and edible mushroom production.

Quorn™ myco-protein

The use of abundantly available waste starch and sugars as a source of raw material for an SCP process was considered in the early 1960s by Lord Rank (then the chairman of the Rank Hovis McDougall (RHM) group of companies) to be a feasible and worthwhile project to alleviate the then anticipated world protein famine. Under the direction of the late Professor Gerald Solomons, a 'starch into protein' process was commenced, which ultimately was to become the only successful SCP process developed entirely with human consumption as the primary aim. Three criteria were considered for this proposed food:

- it must be 'delicious' to eat
- the final product, the substrate and all possible intermediates used in the process must be safe to eat
- above all, the final food presented should be highly nutritious.

Because the final product would require to be textured, bacteria would be unsuitable and filamentous fungi became the obvious choice. While starch was the best available substrate it was considered that for good con- trolled fermentation it would be necessary to use a soluble carbohydrate (glucose) produced by the hydrolysis of starch;

after much experimentation the fungus finally chosen was Fusarium venenatum and this was followed by an extensive fermentation programme that ultimately led to the develop- ment of a novel continuous process.

Edible mushroom production

The world has an immense lignocellulosic sustainable biomass resource. As discussed elsewhere in this volume, intense efforts are in process worldwide to utilise lignocellulose for energy generation. However, one of the most economically viable processes for the conversion of these lignocellulosic

wastes (such as wood and straws) is the cultivation of edible basidiomycete mushrooms.

The cultivation of the common white mushroom, Agaricus bisporus, has expanded worldwide and the USA continues to be the world's largest pro- ducer. However, mushrooms traditionally grown in the Far East, such as Lentinula edodes (the Shiitake mushroom) and Pleurotus spp. (the oyster mush- room), are now expanding into other areas of the world, largely because of their unique flavours and textures and recognised medicinal qualities.

6. Briefly write a note on bio-pesticides .Explain its types and justify bio -pesticides are environmentally friendly.(NOV 2014, NOV 2018).

Definition of Pesticides

The Food and Agriculture Organization (FAO) has defined pesticide as:

any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests

in or on their bodies.

Types of Pesticides

These are grouped according to the types of pests which they kill:

Grouped by Types of Pests They Kill

1. Insecticides – insects
2. Herbicides – plants
3. Rodenticides – rodents (rats & mice)
4. Bactericides – bacteria
5. Fungicides – fungi
6. Larvicides – larvae

Based on how biodegradable they are:

Pesticides can also be considered as:

- Biodegradable:
- The biodegradable kind is those which can be broken down by microbes and other living beings into harmless compounds.
- Persistent:
- While the persistent ones are those which may take months or years to break down.

Another way to classify these is to consider those that are chemical forms or are derived from a common source or production method.

Chemically-related pesticides:

- Organophosphate:

  Most organophosphates are insecticides, they affect the nervous system by disrupting the enzyme that regulates a neurotransmitter.

- Carbamate:

  Similar to the organophosphorus pesticides, the carbamate pesticides also affect the nervous system by disrupting an enzyme that regulates the neurotransmitter. However, the enzyme effects are usually reversible.

- Organochlorine insecticides:

  They were commonly used earlier, but now many countries have been removed Organochlorine insecticides from their market due to their health and environmental effects and their persistence (e.g., DDT, chlordane, and toxaphene).

- Pyrethroid:

  These are a synthetic version of pyrethrin, a naturally occurring pesticide, found in chrysanthemums(Flower). They were developed in such a way as to maximise their stability in the environment.

- Sulfonylurea herbicides:

  The sulfonylureas herbicides have been commercialized for weed control such as pyrithiobac-sodium, cyclosulfamuron, bispyribac-sodium, terbacil, sulfometuron-methyl Sulfosulfuron, rimsulfuron, pyrazosulfuron-ethyl, imazosulfuron, nicosulfuron, oxasulfuron, nicosulfuron, flazasulfuron, primisulfuron-methyl, halosulfuron-methyl, flupyrsulfuron-methyl-sodium, ethoxysulfuron, chlorimuron-ethyl, bensulfuron-methyl, azimsulfuron, and amidosulfuron.

- Biopesticides:

  The biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals.

Examples of pesticides

Examples of pesticides are fungicides, herbicides, and insecticides. Examples of specific synthetic chemical pesticides are glyphosate, Acephate, Deet, Propoxur, Metaldehyde, Boric Acid, Diazinon, Dursban, DDT, Malathion, etc.

Benefits of Pesticides

The major advantage of pesticides is that they can save farmers. By protecting crops from insects and other pests. However, below are some other primary benefits of it.

- Controlling pests and plant disease vectors.
- Controlling human/livestock disease vectors and nuisance organisms.
- Controlling organisms that harm other human activities and structures.

Effects of Pesticides

- The toxic chemicals in these are designed to deliberately released into the environment. Though each pesticide is meant to kill a certain pest, a very large percentage of pesticides reach a destination other than their target. Instead, they enter the air, water, sediments, and even end up in our food.
- Pesticides have been linked with human health hazards, from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm.
- The use of these also decreases the general biodiversity in the soil. If there are no chemicals in the soil there is higher soil quality, and this allows for higher water retention, which is necessary for plants to grow.

4 Eco friendly pest control methods.

1. Plant Your Pest Control in Your Garden

- Mint, basil, lavender, rosemary and other herbs work really well as a natural pest control. Planting these in your garden will not only help keep pests away, but these herbs can be used to control pests indoors as well.
- Herbs are what many consider to be “companion plants” for use in vegetable

gardens and throughout the yard. Their purpose is two-fold: attract beneficial insects and deter pests.

- If you don't want to have companion plants in your garden, yet still want to prevent pests from overtaking the yard, try planing some of these: petunias, chrysanthemums, lemongrass, clover, eucalyptus, lavender or marigolds.

2. Season Your Window Sills

- While most people season their food to make it taste better, certain types of seasoning are known to be quite offensive to insects and bugs.

- Cinnamon, paprika, cayenne, salt, tumeric, black pepper and other seasonings work very well as pest deterrents. Just sprinkle some seasoning in a line along the window sill. Let's say it's your way of drawing a line in the sand.

- You also might want to put some cinnamon, bay leaves or cloves in small bags to place in cupboards, closets, bookshelves or other places around the house where you've found pests.

3. Clean with Vinegar and Essential Oils

- Cleaning is one of the most important steps in the pest prevention process. Rather than bringing in toxic-laden or chemical-filled cleaners, we recommend you do most of your cleaning with natural products.

- One of the simplest cleaners you can make at home, that doubles as a pest deterrent, is 1/2 cup vinegar, 2 cups water and 10-15 drops of essential oils (particularly peppermint or eucalyptus). You can also try a straight-up solution of one part coconut oil and two parts distilled vinegar for a heartier spray.

- If you don't have any essential oils, no worries. You can add citrus peels to a

glass container or jar, then cover with vinegar and let it sit in a cool, dry space for a few weeks. Afterwards you can strain the solution out, add some water and use it as a natural, DIY cleaner and bug repellent.

- Cleaning with vinegar and/or essential oils removes food remnants and smells so bugs, insects and creepy crawlies won't find a reason to stay.

- Regularly wiping down window sills and frames with eucalyptus oil or clove oil is another way to eliminate ant trails and keep pests out. For spiders, you might want to try a garlic spray. Start by blending 10-15 garlic cloves with 1 1/2-2 cups of water. Strain the mixture to remove any pulp, then combine with about a gallon of water. Transfer the garlic spray into a spray bottle and spray it in areas where you've seen spiders. They're not fond of garlic, so this makes an effective pesticide. If you don't want to make such a big batch, you can crush garlic cloves and add them directly to a spray bottle filled with water.

4. Use Food Waste to Turn Pests Away

- Instead of throwing away those orange peels, coffee grounds and cucumber peels, why not put this food waste to good use?

- In addition to using orange, lemon and lime peels to create a citrus cleaning spray, these peels (and even banana peels) can be cut up and added directly to your garden to help fertilize your plants and act as a natural insecticide. Citrus peels can also be placed on counters and window sills to keep pests away. Leftover onion should be chopped up, added to water and placed out in the open to naturally repel mosquitoes.

- Ground coffee lets off a very strong scent. It is even stronger once the coffee grounds are brewed. Fortunately, many pests don't like this smell. So, instead of throwing those grounds away, you can use them to help repel ants, mosquitoes, wasps, bees and another insects. The grounds can be placed

along the outside of your home or on the inside along window sills. (Personally, I prefer outside.)

- Ants do not like cucumbers. The next time a recipe calls for cucumber, or you just want to munch on a cucumber, take the peel and place it in key “points of entry” around your kitchen to help turn pests away. NOTE: Bitter cucumbers, like the slicing cucumber, generally work best.

- Side note: you should also consider storing food in glass or stainless steel, pest-proof containers. This will help preserve food, prevent waste and make sure you’ve got nothing for those pesky pests to find.

7.Write a detailed note on biopolymers.(NOV 2015, NOV 2016)

DEFINITON

- Biopolymers are naturally occurring polymers, which are produced by living organisms. They are distinct from synthetic biodegradable polymers.

- There has been growing concern about the negative impacts of environmental pollution from fossil fuels and waste from petrochemical products. A lot of research has gone into exploring other alternatives to petroleum-based products which would be renewable as well as bio-degradable and thus pose a lesser risk to the environment.

- Biopolymers are one such possible solution to the problem because they are typically biodegradable materials obtained from renewable raw materials. However, it must be noted that not all biodegradable polymers are biopolymers (i.e. produced from renewable resources). As one might expect, there are challenges related to biopolymers such as their limited rate of production, cost of production and the suitability of their properties.

- Some of the first modern biomaterials made from natural biopolymers

include rubber, linoleum, celluloid and cellophane. The latter two are made using cellulose, which is the most naturally abundant biopolymer and the most abundant organic material on Earth, making up a third of all plant matter. Since the middle of the 20th century, these human-made biopolymers were virtually all replaced with petrochemical-based materials. However, due to growing ecological concerns, biopolymers are enjoying renewed interest from the scientific community, the industrial sector and even in politics .

- The properties of biopolymers
- The production and processing of biopolymers
- Applications of biopolymers
- Examples of biopolymers
- The future of biopolymers

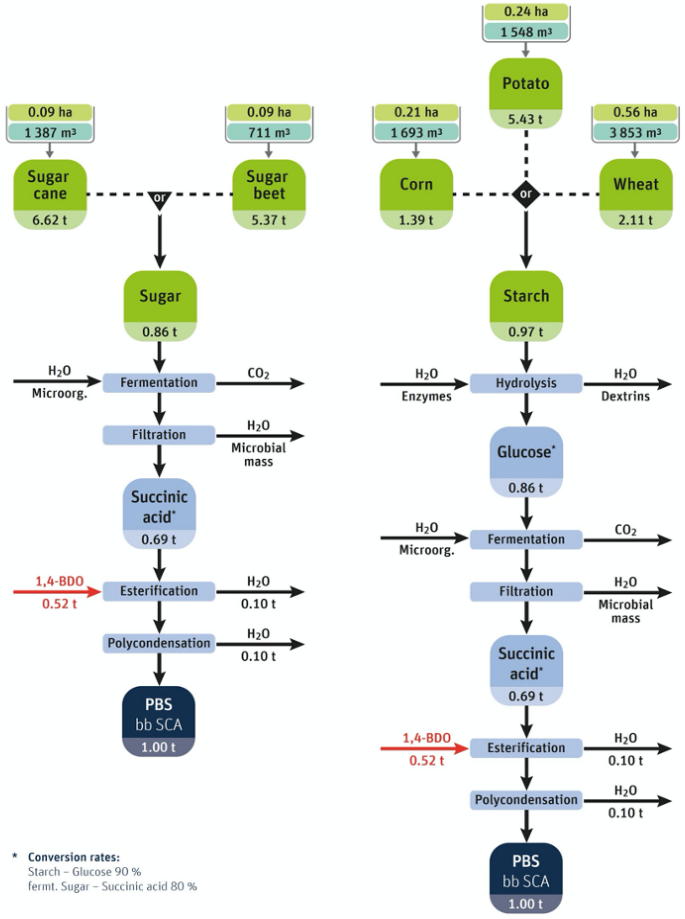
Properties of biopolymers

- ❖ The main interest in biopolymers is to replace many of the everyday items which are made from petroleum products. This means that they will be required to exhibit similar, if not better, properties than the materials they replace to make them suitable for the various applications that they will be put to. Much of the property measurements of biopolymers have variance due to factors such as degree of polymerisation, type and concentration of additives, and presence of reinforcement materials. Information about the properties of biopolymers is not as extensive as for traditional polymers, but there is still a considerable depth of investigation into their physical, mechanical, thermal properties [2].
- ❖ Some biopolymers have been identified to possess electronic and ionic conductivity and have thus been termed electro-active biopolymers (EABP). This has given them the potential to replace other synthetic materials. These biopolymers, which include starch, cellulose, chitosan and pectin, show a wide -ranging electrical conductivity between 10-3 and 10-14 S/cm [3].

The production and processing of biopolymers

There are many different methods and techniques used to produce biopolymers.

Since most of these polymers already exist in nature or are produced by natural organisms, these processes are often a matter of extraction followed by synthesis. They may include a combination of any of fermentation, filtration, compounding/granulation, hydrolysis, esterification, poly-condensation, oxidation and dehydration. Below is an example of the production process involved in making polybutylene succinate (PBS).



rocess route for the production of polybutylene succinate (PBS) with bio-based succinic acid (PBS bb SCA).

Applications of biopolymers

- Biopolymers are used in many industrial applications as well as food packaging, cosmetics and medicine [4]. They can replace traditional petroleum-based plastics in many applications. Some biopolymers have also

been applied to specific uses that other plastics would not be suitable for, such as in the creation of artificial tissue. These applications may require biocompatible and biodegradable materials with sensitivity to changes in pH as well as physicochemical and thermal fluctuations [5].

- Biopolymers, in general, often exhibit poor mechanical properties, chemical resistance and processability in comparison to synthetic polymers. To make them more suitable for specific applications, they can be reinforced with fillers which drastically improve these properties. Biopolymers that have been reinforced in this way are called biopolymer composites. The table below is a summary of some common biopolymer composites, their properties and the industries in which they are already widely used.

Summary of biopolymer composites production methods, properties, and applications.

Matrix/Filler	Production Method	Properties	Applications
PLA/PEG/Chit	Extrusion	Low stiffness/ High flexibility	Bone & dental implants food packaging
PLA/Cellulose	Extrusion/injection	Improved rigidity & biodegradability	Packaging, automotive
PLA/Potato pulp	Extrusion/injection	Low stiffness & ductility, good processability	Food packaging

PLA/MgO	Solution casting	Improved stability and bioactivity	Medical implants, tissue engineering, orthopaedic devices
PHB/wood sawdust fibres	Extrusion	Improved degradation in soil	Agriculture or plant nursery
PHBV/TPU/cellulose	Extrusion/injection	Balanced heat resistance, stiffness, and toughness	Food packaging tissue engineering
Nanocellulose/CNT	Cast moulding	Good electrical conductivity	Super-capacitor, sensors
Rubber/potato starch	Roller mixing	Accelerated thermal ageing	Vibration isolators, shock mounts, electrical components
Potato starch/wheat gluten	Compression moulding	Improved maximum stress & extensibility	Development of bio-based plastics

Alginate/cinnamon oil	Solution casting	Good antibacterial activity	Active packaging materials
PVA/Chitosan	Electro-spinning	Good chemical stability	Drug delivery food packaging
PPC/TPU	Melt compounding	Good thermal stability & stiffness	Electronic packaging applications

Examples of biopolymers

Biopolymers can be classified broadly into three categories based on their monomeric units and structure:

- Polynucleotides: DNA (deoxyribonucleic acid) and RNA (ribonucleic acid)
- Polysaccharides: cellulose, chitosan, chitin, etc.
- Polypeptides: collagen, gelatin, gluten, whey, etc.

Biopolymers can also be categorised by other criteria such as their base materials (animal, plant or microbial), their biodegradability, their synthesis route, their applications or their properties.

Examples of some commercially-produced biopolymers include [1]:

- Bio-based polyesters such as polylactic acid (PLA), polyhydroxybutyrate (PHB), polybutylene succinate (PBS), polybutylene succinate adipate (PBSA), polytrimethylene terephthalate (PTT)
- Bio-based polyolefins such as polyethylene (Bio-PE)
- Bio-based polyamides (Bio-PA) such as homopolyamides (Bio-PA 6, Bio-PA 11) and copolyamides (Bio-PA 4.10 – Bio-PA 5.10 – Bio-PA 6.10, Bio-PA 10.10)
- Polyurethanes such as Bio-PUR

- Polysaccharide polymers such as cellulose-based polymers (regenerated cellulose, cellulose diacetate) and starch-based polymers (thermoplastic starch, starch blends) The future of biopolymers

  The figure below shows the increase in bio-based polymer production between 2017 and what is estimated to be the case in 2022. Furthermore, it is projected that biodegradable biopolymers will constitute a larger percentage of biopolymer production in the coming years. It is clear to see that biopolymer production is on an upward trajectory. While it has a long way to go, if it is to take over from petroleum products, production is forecasted to increase from 2.27 million tonnes in 2017 to 4.31 million tonnes in 2022. This is at least partly a result of public demand and government regulations, which will continue to have a significant impact.