MODULE 2: BIOMOLECULES AND THEIR APPLICATIONS

- **Carbohydrates** (cellulose-based water filters, PHA and PLA as bioplastics),
- **Nucleic acids** (DNA Vaccine for Rabies and RNA vaccines for Covid19, Forensics – DNA fingerprinting),
- **Proteins** (Proteins as food – whey protein and meat analogues, Plant-based proteins),
- **Lipids** (biodiesel, cleaning agents/detergents),
- **Enzymes** (glucose-oxidase in biosensors, lignolytic enzyme in bio-bleaching).

Introduction

Biomolecules: Biomolecules are the molecules in all living organisms involved in the maintenance and metabolic process.

Classification: **Biomolecules are classified majorly into two types.**

1. ***Inorganic:*** Elements other than carbon, hydrogen, oxygen, nitrogen sulphur and phosphorous are considered inorganic biomolecules. They are much needed for structural support, transportation, and many other functions of living beings.
2. ***Organic:*** Molecules mainly made of carbon hydrogen, oxygen, nitrogen sulphur and phosphorous and posing numerous roles in living beings are called organic biomolecules.

These organic biomolecules are classified into 6 major categories ***Carbohydrates, Lipids, Proteins, Nucleic Acids, Enzymes, and Vitamins.*** These are very large molecules of many atoms which are covalently bonded. Energy is stored in covalent bonds. When we eat, we get the energy to live because chemical reactions within our bodies break these bonds.

CARBOHYDRATES

Carbohydrates: ***"Carbohydrate is the class of biomolecules consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen–oxygen atom."***

- Carbohydrates provide energy to our body and are the best fuel source for the body.
- Carbohydrates also help to digest protein and fat.

"Cellulose is an abundant complex carbohydrate, or polysaccharide, consisting of 3,000 or more glucose units. They are present in a Plant's cell wall, insoluble in water, and biodegradable".

Cellulose-based water filters.

These water filters are made from cellulose; they can remove impurities and contaminants from water and are an alternative to traditional synthetic polymer filters. On treating with required antimicrobial agents, the cellulose filters can effectively remove pathogens, and other contaminants from water, making it safer and more potable.

Construction of cellulose-based water filters

1. **Cellulose Material Selection:** The type of cellulose material used in the water filter will depend on the desired properties, such as strength, porosity, and chemical resistance. Common cellulose materials include paper, cotton, and wood fibers.
2. **Cellulose Preparation:** Materials are fragmented into small pieces, washed to remove impurities, and dried.

3. **Cellulose Layer Formation:** The cellulose material is made into a layer by either **stacking** or **compacting** it using **heat & pressure.**
4. **Filter Medium Attachment:** The cellulose layer is attached to a filter medium, such as a mesh or a support structure, to provide stability and increase the filter surface area.
5. **Chemical Treatment:** The cellulose layer may be chemically treated to modify its properties, such as increasing its hydrophilicity or adding antimicrobial agents.
6. **Filter Testing:** The completed filter is tested to ensure it meets the desired specifications, such as filtration efficiency & flow rate.

Properties of cellulose-based water filter

1. **High Porosity:** They have a highly porous structure allowing them to remove impurities and contaminants efficiently from water.
2. **Biodegradability:** Cellulose-based water filters are biodegradable, reducing the environmental impact compared to synthetic polymer filters.
3. **Cost-effective:** Cellulose-based water filters are less economical than traditional synthetic polymer filters.
4. **Renewable resource:** Cellulose-based water filters are made from a renewable resource; this reduces the dependency on non-renewable resources.
5. **Good mechanical strength:** They pose good mechanical strength, allowing them to maintain their structure and perform effectively over time.
6. **Chemical resistance:** Cellulose-based water filters resist most chemicals, including acids and bases, and hence can be used in various water treatment applications.
7. **Large surface area:** Cellulose-based water filters have a large surface area, enhancing filtration capabilities and reducing filter replacement frequency.

8. Advantages and Limitations of cellulose-based water Filter.

Advantages	Limitation
Environmentally friendly.	Low resistance to high temperature.
Cost-effective.	Low filtration efficiency for certain contaminants.
Designed in any shape and size.	Difficult to sterilize and clog easily.
Chemically resistant.	Affect the taste of water.
Have a high porosity structure, thus effective removal of impurities.	Need replacement more frequently due to its limited lifespan.

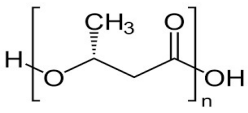
Polyhydroxyalkanoates and Polylactic Acid as Bioplastic

Polyhydroxy-alkanoates (PHAs) and Polylactic acids are biodegradable polyesters produced by microorganisms, such as bacteria and fungi. They are made from renewable resources, such as sugar and cornstarch, and are an environmentally friendly alternative to traditional petroleum-based plastics.

***Polyhydroxyalkanoates* (PHAs)**

Industrial production of PHA

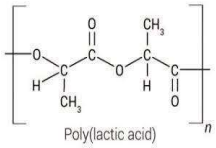
- The PHA is extracted and purified from the bacteria by microbial fermentation of sugar, glucose, or vegetable oil in an optimized condition.
- PHA is used for preparing either thermoplastic or elastomeric materials, with melting points ranging from 40 to 180 °C.



Polylactic Acid (PLA)

Industrial production of PLA

- PLA are bioplastics produced by condensation polymerization of lactic acid obtained by the fermentation of corn starch, sugarcane, or other carbohydrate resources.
- Its low melting point, high strength, low thermal expansion, good layer adhesion, and high heat resistance make it an ideal material for 3D printing.



Properties and application of PHA and PLA

Properties	Applications
Biodegradability: Can break down into water and carbon	**Packaging:** Food containers, beverage cups, and

dioxide, reducing their environmental impact.	clamshell containers
Renewable: Used in medical devices due to its zero adverse reactions in the body.	**Medical Devices:** In medical devices such as sutures, implants, and drug delivery systems
Stiffness and Strength: PLA has good stiffness and strength but is not as strong as traditional petroleum-based plastics	**Agricultural Mulch Films:** Biodegradable mulch films for agriculture to reduce soil erosion and conserve moisture.
Clear/Transparent: PLA has a clear and transparent appearance.	**Consumer Goods:** Consumer goods, such as toys, phone cases, and water bottles.
Biocompatible: No adverse effect hence can be used in medical applications.	**Electronic parts:** In biodegradable components in electronic devices such as smartphones and laptops.
Mechanical properties: Similar mechanical properties as traditional petroleum-based plastics.	**Aerospace:** In aerospace applications, such as insulation and cable management.
Printability: Used in 3D printing due to its good printability and ease of use.	**Sporting Goods:** Biodegradable sporting goods such as golf tees and fishing lures.
Processing: It can be processed using conventional plastic processing techniques, such as injection moulding, blow moulding, and extrusion	**Construction:** Biodegradable insulation and soundproofing materials.

Nucleic Acids

Nucleic Acids are biopolymers crucial in storing and transferring genetic information in all living organisms. Nucleic acids are of two types.

Deoxyribonucleic acid (DNA)	Ribonucleic acid (RNA)
DNA is the genetic material that guides all living organisms' development, functioning, and reproduction.	RNA is critical for the transmission of the genetic code that is necessary for protein creation from the nucleus to the ribosome.
DNA is a double-stranded helix structure composed of • Sugar (deoxyribose), • Phosphate group, and • Nitrogenous base (adenine, guanine, cytosine, or thymine). 	**RNA is a single-stranded molecule** composed of • sugar (ribose), • Phosphate group, and • Nitrogenous base (adenine, guanine, cytosine, or uracil). 

DNA replicates on its own, it is self-replicating.	RNA does not replicate on its own. It is synthesized from DNA when required.

DNA Vaccines

DNA Vaccines are third-generation vaccines that use engineered **viral or bacterial DNA** to stimulate an immune response in the body against a specific pathogen or cancer cell. The goal of DNA vaccines is the same as that of traditional vaccines, but they work slightly differently.

DNA Vaccines for Rabies

Rabies is a preventable viral disease most often transmitted through the bite of a rabid animal. The rabies virus infects the central nervous system of mammals, ultimately causing disease in the brain and death.

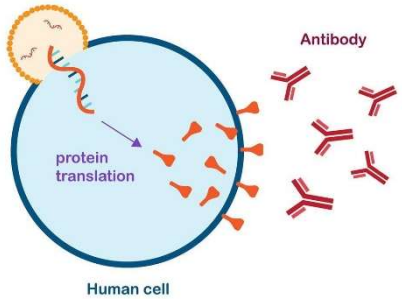
1. DNA of the rabies virus carrying the genetic information (gene of interest) is cut and introduced into the plasmids (circular DNA found in microorganisms).
2. These engineered plasmids are introduced into mammals which enter the cells. This RNA produces harmless viral proteins which reach the surface of the cells.
3. This triggers the generation of an immune response (antibodies) system within the body of mammals.
4. This information about antigens is stored inside the body, whenever rabies viruses enter the body, the body produces antigens to fight against these viruses.

Advantages and disadvantages of DNA Vaccines

Advantages	Disadvantages
• **Efficacy:** DNA vaccines effectively prevent rabies infection in animal and human trials. • **Long-lasting protection:** Protect against rabies for extended periods. • **Ease of administration:** via injection or even delivered orally. • **Cost-effective:** DNA vaccines are relatively inexpensive compared to traditional vaccines. • **Safe and low side effects:** DNA vaccines do not contain live virus particles, making them safer and less.	• The risk of affecting genes that control cell growth. • Repeated doses are required. • Lower immunogenicity than an inactivated vaccine. • Limited to protein antigens. • No mass application for animals, etc.

RNA Vaccines for COVID-19

1. The RNA of the COVID-19 virus is introduced into the body; this enters the cells and produces harmless viral proteins that reach the cells' surface.
2. This triggers the generation of an immune response (antibodies) system within the body.
3. Since this information about antigens is stored inside the body, whenever the COVID-19 viruses enter the body, the body produces antibodies to fight against these viruses.



Advantages and disadvantages of RNA Vaccines

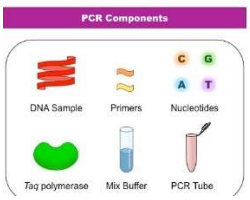
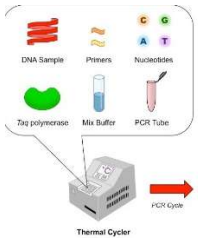
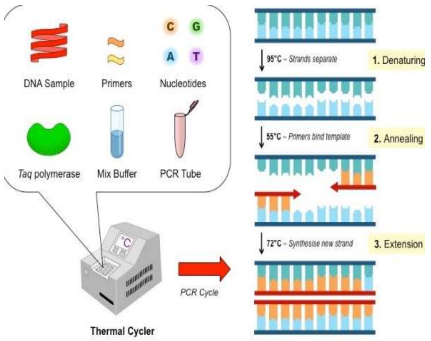
Advantages	Disadvantages
• **Safety:** these are not made with pathogen particles or inactivated pathogens; hence are non-infectious. It will not integrate itself into the host genome, and the RNA strand in the vaccine is degraded once the protein is made. • **Efficacy:** these vaccines generate a reliable immune response & are well-tolerated by healthy individuals, with few side effects. • **Production:** vaccines can be produced more rapidly in the laboratory in a process that can be standardized.	• **Unintended effects:** the mRNA strand in the vaccine may elicit an unintended immune reaction. • **Delivery:** delivering the vaccine effectively to cells is challenging since free RNA in the body is quickly broken down. • **Storage:** needs to be frozen or refrigerated.

DNA Fingerprinting

DNA Fingerprinting, DNA profiling, or genetic fingerprinting, is a technique used in forensic science to identify an individual based on their unique DNA profile. The process involves analyzing specific regions of an individual's DNA, called markers, which can vary from person to person.

Working on DNA fingerprinting for forensic applications

1. **Sample collection:** DNA is extracted from a biological sample, such as **blood, skin, or hair**. The sample is then purified and processed to isolate the DNA.
2. **DNA amplification:** The extracted DNA is amplified using a **Polymerase Chain Reaction (PCR)** technique.
3. **DNA analysis:** The amplified DNA is then analyzed using **Gel Electrophoresis.** The DNA fragments are separated based on size and charge, and a DNA profile is generated.
4. **DNA comparison:** The DNA profile obtained from the biological sample is then compared to the DNA profiles of other individuals, such as suspects or victims, to determine if there is a match.

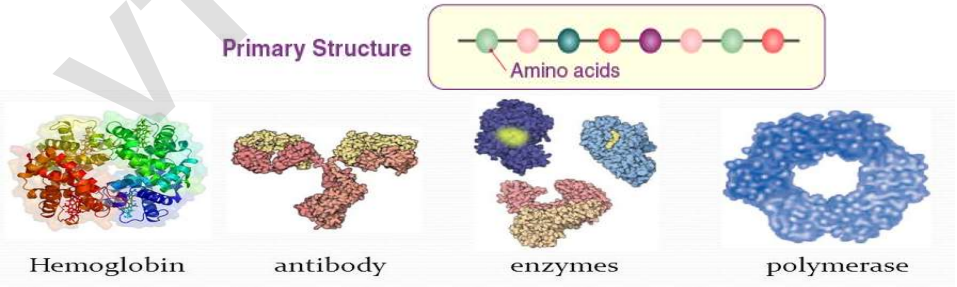
Step 1:	Step 2:	Step 3:	Step 4:
			

Advantages and disadvantages of DNA Fingerprinting

Advantages	Disadvantages
• **Unobtrusive form of testing:** DNA can be found in many body fluids and tissues; retrieving materials is simple. • **Used for more than criminal justice purposes** be used to create genetic profiles for suspects and can help people research their ancestry. • **Collected evidence can be stored indefinitely:** DNA samples do not degrade over time like other forms of forensic evidence & hence can be stored for longer periods. • **Identify hereditary diseases:** used to identify certain hereditary diseases that may be life-threatening if not discovered immediately, e.g., phenylketonuria.	• **Creates privacy issues.** • **Accuracy of DNA fingerprinting is overly influential.** • It may show the risks of developing cancer, obesity, or other health problems over time. This information could be used to exclude people from receiving certain medical coverage

Proteins

Proteins are large, complex molecules made up of chains of smaller building blocks called amino acids. Proteins are essential nutrients that provide the body with amino acids, which are the building blocks of the body's tissues. They perform a wide range of bodily functions, including catalyzing biochemical reactions, transporting materials like haemoglobin, providing mechanical support like muscles, keratin, and many others related to body functioning. These proteins of formed by the sequential arrangement of amino acids (building blocks of proteins). The specific sequence of amino acids in a protein determines its unique structure and functions of the proteins.



Proteins as food – whey protein

Whey protein is a type of protein derived from the liquid that separates from milk during the cheese-making process. Compared to other types of protein, whey protein is high in branched-chain amino acids, which are essential for muscle growth and repair and are rapidly absorbed by the body; hence it is widely used as a dietary supplement, particularly by athletes, bodybuilders, and people looking to increase their protein intake.

Use of whey protein as food: Whey protein is derived from cow's milk and commonly used as a supplement. There are several uses of whey protein as food, including.

- **Sports nutrition:** Athletes and fitness enthusiasts often use whey protein to help build and repair muscle tissue, support recovery after intense exercise, and increase overall muscle mass.
- **Weight management:** It can be used to help manage weight.
- **Health promotion:** Whey protein is rich in essential amino acids and has been shown to have various health benefits, including improved immune function, lower blood pressure, and reduced risk of cardiovascular disease.
- **Meal replacement:** Whey protein can be used as a meal replacement, as a drink or in various food products. It provides a quick and convenient source of protein, making it a popular option for people with busy schedules or limited access to fresh foods.

Proteins as food : Meat analogues of protein

Meat analogues, or meat substitutes or alternatives, are plant-based foods designed to mimic meat's taste, texture, and appearance. They are made from various ingredients, including soy protein, wheat protein, pea protein, and other plant-based ingredients.

Some important meat analogues are Tofu, Tempeh, Seitan, Veggie burgers, Meatless meatballs, Plant-based sausages, and others. These meat analogues provide a similar taste, texture, and nutritional profile to meat, making it easier for people to reduce or eliminate their me at consumption for health or ethical reasons.

Plant-based proteins are derived from plant sources, such as legumes, grains, nuts, and seeds. Used as an alternative to animal-based proteins, especially for those following a vegetarian or vegan diet.

- **Benefits of plant-based proteins:**
- **Sustainable:** Plant-based protein sources are more environmentally sustainable than animal-based sources.
- **Eco-friendly:** requires fewer resources to produce, generating fewer greenhouse gas emissions.
- **Nutrient-rich:** Many plant-based protein sources also contain other essential nutrients, such as fiber, vitamins, and minerals.
- **Hypoallergenic:** Plant-based proteins are a good option for people with food allergies or sensitivities.
- **Cost-effective:** Plant-based protein sources are often more affordable than animal-based sources.

Proteins as food : plant-based proteins

Definition of a plant-based protein. A plant-based diet consists of all minimally processed fruits, vegetables, whole grains, legumes, nuts and seeds, herbs, and spices and excludes all animal products, including red meat, poultry, fish, eggs, and dairy products.

Benefits of plant-based proteins:

- **Sustainable**: Plant-based protein sources are more environmentally sustainable than animal-based sources.
- **Eco-friendly**: requires fewer resources to produce, generating fewer greenhouse gas emissions.

- **Nutrient-rich**: Many plant-based protein sources also contain other essential nutrients, such as fiber, vitamins, and minerals.
- **Hypoallergenic**: Plant-based proteins are a good option for people with food allergies or sensitivities.

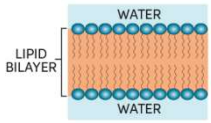
Uses of plant-based proteins:

- **Dietary supplements:** Plant-based proteins are sold as powders, bars, etc. A convenient way to add protein to a diet.
- **Food products:** Plant-based proteins are used as ingredients in a variety of food products, including plant-based meat analogs, protein bars, and smoothies.
- **Health and wellness:** Plant-based proteins are often marketed as a healthier alternative to animal-based proteins.
- **Vegetarian and vegan diets:** Plant-based proteins are a popular source of protein for people following a vegetarian or vegan diet.
- **Fitness and sports nutrition:** Plant-based proteins are also used by athletes and fitness enthusiasts to support muscle recovery and growth.

Lipids

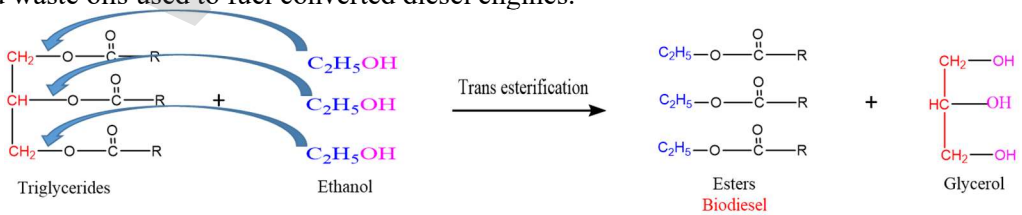
Lipids are a group of organic compounds that include fats, oils, waxes, and some hormones. Lipids are a major source of stored energy in the body, and they can be broken down to release energy when it is needed.

- Lipids have hydrophobic (Tail part), nonpolar molecules. And hydrophillic (Head part) polar (water) and are soluble in nonpolar solvents.
- They are isolated from the other biological molecules by extracting.



Lipids as Biodiesel

Biodiesel is generally made by esterifying lipids (e.g., soybean oil, vegetable oil, and animal fat) with alcohol-generating fatty acid esters. Biodiesel is suggested to be utilized in standard diesel engines and is thus well defined from the vegetable and waste oils used to fuel converted diesel engines.



Process of Obtaining Biodiesel from Lipids

1. **Raw material preparation:** The lipids (animal fats, Vegetable oils) are collected and purified.
2. **Transesterification:** The purified lipids are mixed with alcohol and a catalyst to produce **fatty acid methyl esters (FAME),** the main components of biodiesel. This process is known as **transesterification.**
3. **Separation:** The reaction mixture is then separated into two layers: the upper layer contains the FAME (biodiesel) and the lower layer contains the glycerol (byproduct).
4. **Purification:** The biodiesel is washed with water to remove excess alcohol and further purified to remove impurities and improve quality.

Lipids as Cleaning agent / Detergents

Lipids have several properties that make them suitable as cleaning agents, including their ability to emulsify and dissolve grease and oils. Additionally, lipids can form micelles, which are tiny spherical structures that can surround and trap dirt particles, making it easier to remove them; hence they are used to manufacture soap, detergents, personal care products, and others.

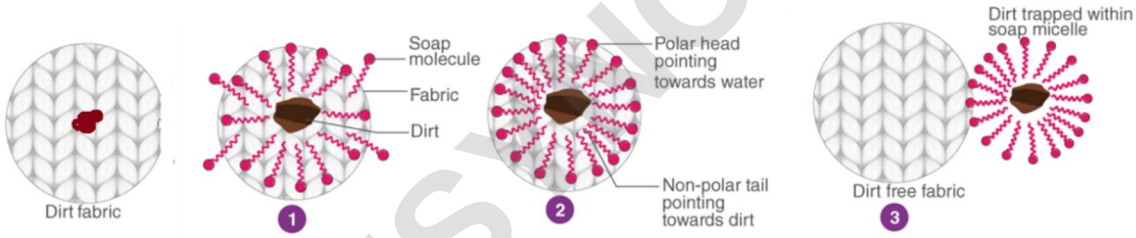
Working principle of lipids as a cleaning agent

The dirt present on clothes is organic in nature and insoluble in water. Therefore, it cannot be removed by only washing with water.

Step 1: When Lipid detergent is dissolved in water, its hydrophobic Tail ends attach to the dirt.

Step 2: Then the molecules of Lipid detergent arrange themselves in micelle formation and trap the dirt at the center of the cluster.

Step 3: These micelles remain suspended in the water. The dust particles are then easily rinsed away by water.



Advantages of lipids as cleaning agents/detergents

- **Biodegradability**: Lipids are derived from natural sources, like plants and animals, and are more biodegradable and environmentally friendly than many synthetic cleaning agents.
- **Renewable resources**: Lipids are from renewable resources, such as crops, and are not based on fossil.
- **Effectiveness**: Lipids have excellent grease-cutting properties, making them effective cleaning agents.
- **Mildness**: Lipids are mild and gentle, making them suitable for personal care products (soaps and shampoos).
- **Cost-effective**: Lipids can be less expensive than synthetic cleaning agents.

Enzymes

Enzymes are proteins that act as biocatalysts in biological reactions to speed up the rate of chemical reactions without being consumed in the process. Enzymes are involved in various metabolic processes, digestion, and cellular respiration. All living things have enzymes. Our bodies naturally produce enzymes. But enzymes are also in manufactured products and food.

Properties of Enzymes for Engineering Applications

- **Specificity:** Enzymes are specific in performing specific tasks.
- **Reactivity:** They speed up the rate of chemical reactions without being consumed.
- **Renewability:** Enzymes are biodegradable & obtained from renewable resources,

- **Cost-effectiveness:** Enzymes can be produced in large quantities through fermentation, making them cost-effective.

Engineering Applications of Enzymes

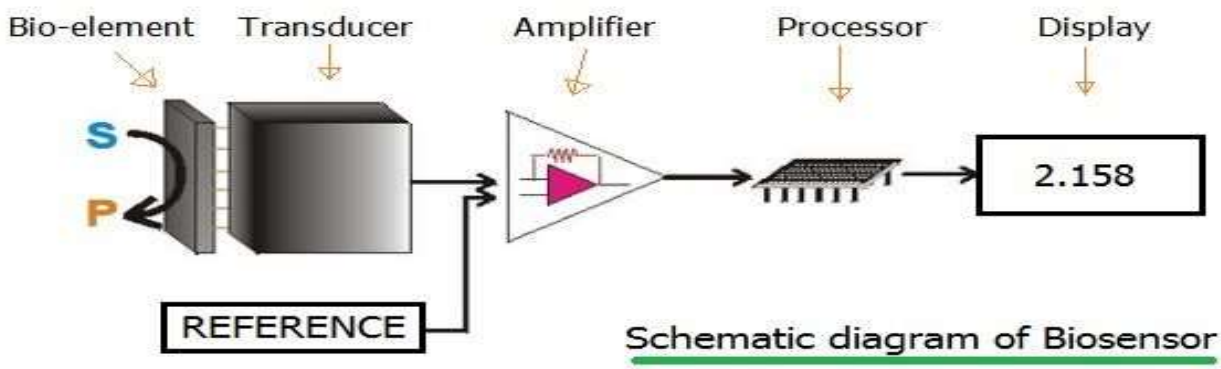
- **Biofuel production:** Enzymes are used to convert plant material into biofuels, such as ethanol and biodiesel.
- **Food and beverage production:** Used in the food and beverage industry such as baking, cheese making, and juice production.
- **Textile production:** Enzymes are used to remove stains, whiten fabrics, and improve the softness of textiles.
- **Pharmaceuticals:** Enzymes are used to produce various pharmaceutical products, such as antibiotics and vaccines.

Enzymes: Glucose-oxidase in biosensors

Enzymes in Biosensors: Biosensors are analytical devices that combine a biological recognition element with a transducer to detect & quantify target analytes.

- The biological recognition element can be an enzyme, antibody, nucleic acid, or other biological molecule that specifically interacts with the target analyte.
- The transducer converts the biological response into an electrical signal that can be quantified and interpreted.

1. Glucose oxidase (GOx) is the enzyme commonly used in biosensors to detect glucose levels in biological fluids, such as blood and urine.
2. GOx is typically immobilized in glucose biosensors on a polymer film. The transducer in the biosensor is an electrode or other type of sensor.
3. The glucose level in the blood can be measured by applying a drop of blood to a chemically treated, disposable **'test strip immobilized with glucose oxidase**, which is then inserted into an electronic blood glucose meter.
4. The **enzyme catalyzes glucose oxidation** to **Gluconic acid**. Which results in electrons removal The meter detects the electrons. The liberated electrons are directly proportional to amount of glucose present in the blood. Which displays in units of mg/dL or mmol/L.

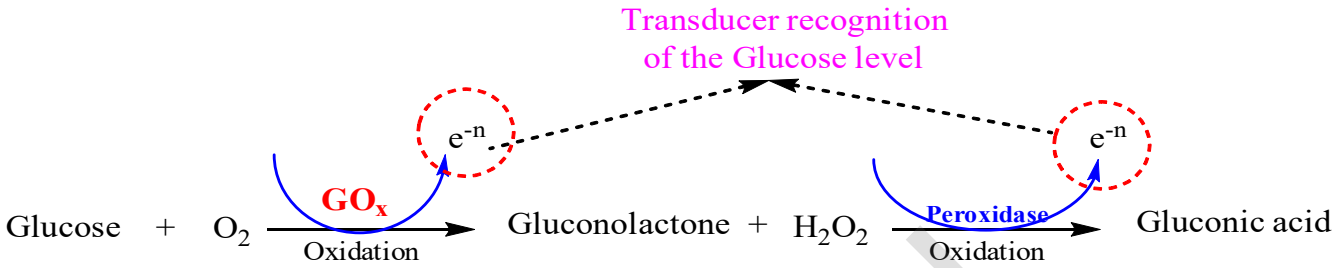


Schematic diagram of Biosensor

The following reaction is involved in this process.

Glucose + O_2 GOx^{-}→Gluconolactone + H_2O_2 (1)

Gluconolactone + H_2O → Gluconic acid (2)



Enzymes: a ligninolytic enzyme in bio-bleaching

Biobleaching, also known as bleaching, is the process of pretreating pulp with enzymes. Biobleaching is achieved using microbial enzymes, ligninolytic. The ligninolytic enzymes are a group of enzymes found in different types of organisms as plants, bacteria, insects, and fungi. The major ligninolytic enzymes are laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase.

Bio-bleaching is a process that uses **biological agents**, such **as enzymes, to remove color** and **brighten fibers**, paper, and textiles. This makes it a more sustainable alternative to **traditional chemical bleaching methods** that use **harsh chemicals** like hydrogen peroxide and chlorine.

1. In bio-bleaching, ligninolytic enzymes play a crucial role in the removal of lignin from pulp fibers, which is an essential step in the production of bleached pulp for papermaking. One of the key ligninolytic enzymes used in bio-bleaching is ***lignin peroxidase (LiP).***
2. In bio-bleaching, **LiP** acts on lignin present in the pulp fibers, breaking down its complex structure.
3. This enzymatic degradation of lignin improves the brightness and color of the pulp, making it suitable for high-quality paper production.
4. LiP achieves lignin degradation through Oxidation: LiP oxidizes lignin compounds resulting in the cleavage of lignin bonds, leading to the breakdown of lignin macromolecules.

5. The combined action of these enzymes can effectively remove lignin, allowing for improved pulp quality and reduced chemical bleaching requirements.

Fig: Application of Lignin

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