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| Name ofUnit: | Introduction to Biotechnology |
| Subject/Course name: | B Pharmacy/ Pharmaceutical Biotechnology |
| Course/ Subject Code: | BP605T |
| Class: B.Pharm. Semester: | 6th |
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## Learning Outcome of Module-1

| LO | Learning Outcome | Course Outcome Code |
|---|---|---|
| **LO1** | Students will be able to understand the basic concept related to biotechnology in pharmaceuticals. | BP605.1 |
| **LO2** | They will be able to learn about the concept of enzyme immobilization. | BP605.1 |
| **LO3** | Students will also be able to know about protein engineering | BP605.2 |
| **LO4** | They will be able to understand the basics of genetic engineering. | BP605.2 |

## Module Contents

| Topic |
| --- |
| • Brief introduction to Biotechnology with reference to Pharmaceutical Sciences.<br>• Enzyme Biotechnology- Methods of enzyme immobilization and applications.<br>• Biosensors- Working and applications of biosensors in Pharmaceutical Industries.<br>• Diffusion principles in biological system.<br>• Brief introduction to Protein Engineering.<br>• Use of microbes in industry. Production of Enzymes- General consideration<br>• Amylase, Catalase, Peroxidase, Lipase, Protease, Penicillinase.<br>• Basic principles of genetic engineering. |

# BIOTECHNOLOGY

The use of biology to solve problems and make useful products. The most prominent area of biotechnology is the production of therapeutic proteins and other drugs through genetic engineering.

Biotechnology is a science-driven industry sector that uses living organisms and molecular biology to produce healthcare-related products. Biotechnology companies also develop therapeutics or processes (such as DNA fingerprinting). Biotechnology is best known for its role in medicine and pharmaceuticals, but the science is also applied in other areas such as genomics, food production, and the production of biofuels.

## History of biotechnology

People have been harnessing biological processes to improve their quality of life for some 10,000 years, beginning with the first agricultural communities. Approximately 6,000 years ago, humans began to tap the biological processes of microorganisms in order to make bread, alcoholic beverages, and cheese and to preserve dairy products. But such processes are not what is meant today by biotechnology, a term first widely applied to the molecular and cellular technologies that began to emerge in the 1960s and ’70s. A fledgling “biotech” industry began to coalesce in the mid- to late 1970s, led by Genentech, a pharmaceutical company established in 1976 by Robert A. Swanson and Herbert W. Boyer to commercialize the recombinant DNA technology pioneered by Boyer, Paul Berg, and Stanley N. Cohen. Early companies such as Genentech, Amgen, Biogen, Cetus, and Genex began by manufacturing genetically engineered substances primarily for medical and environmental uses.

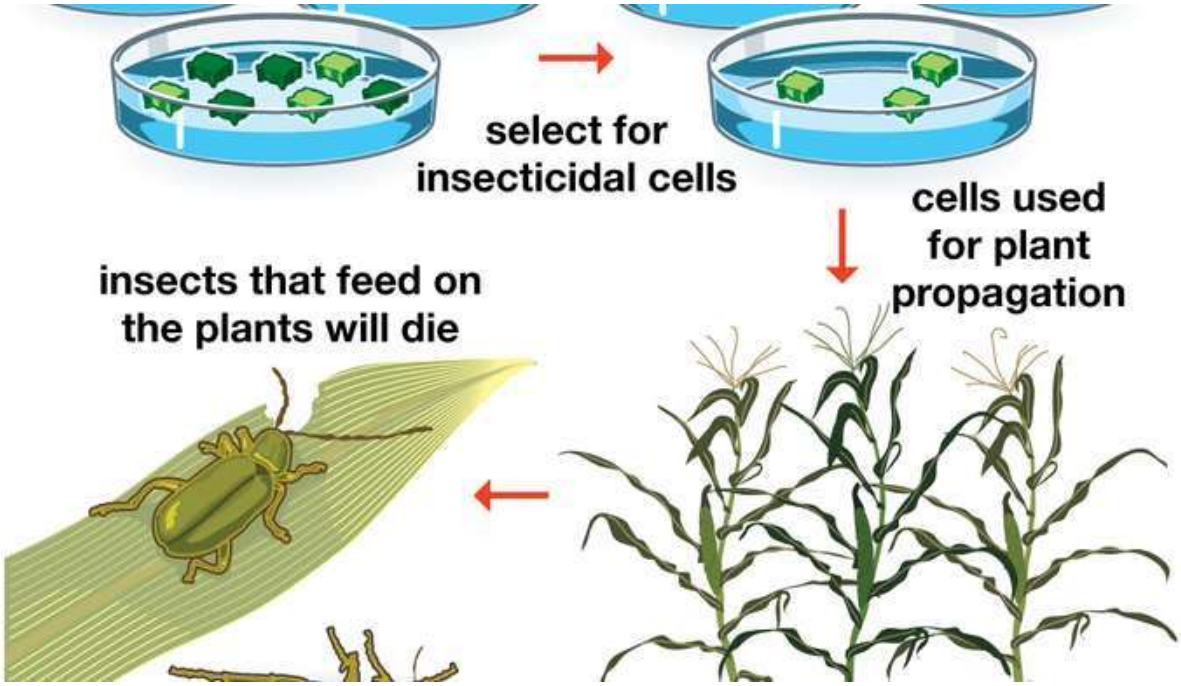
## Applications of biotechnology

Biotechnology has numerous applications, particularly in medicine and agriculture. Examples include the use of biotechnology in merging biological information with computer technology (bioinformatics), exploring the use of microscopic equipment that can enter the human body (nanotechnology), and possibly applying techniques of stem cell research and cloning to replace dead or defective cells and tissues (regenerative medicine). Companies and academic laboratories integrate these disparate technologies in an effort to analyze downward into molecules and also to synthesize upward from molecular biology toward chemical pathways, tissues, and organs.

In addition to being used in health care, biotechnology has proved helpful in refining industrial processes through the discovery and production of biological enzymes that spark chemical reactions (catalysts); for environmental cleanup, with enzymes that digest contaminants into

harmless chemicals and then die after consuming the available “food supply”; and in agricultural production through genetic engineering.

Agricultural applications of biotechnology have proved the most controversial. Some activists and consumer groups have called for bans on genetically modified organisms (GMOs) or for labeling laws to inform consumers of the growing presence of GMOs in the food supply. In the United States, the introduction of GMOs into agriculture began in 1993, when the FDA approved bovine somatotropin (BST), a growth hormone that boosts milk production in dairy cows. The next year, the FDA approved the first genetically modified whole food, a tomato engineered for a longer shelf life. Since then, regulatory approval in the United States, Europe, and elsewhere has been won by dozens of agricultural GMOs, including crops that produce their own pesticides and crops that survive the application of specific herbicides used to kill weeds.




In modern industry natural resins have been almost entirely replaced by synthetic resins, which are divided into two classes, thermoplastic resins, which remain plastic after heat treatment, and thermosetting resins, which become insoluble and infusible on heating.

The genetic industries include agriculture, forestry, and livestock management and fishing all of which are subject to scientific and technological improvement of renewable resources. The extractive industries include the mining of mineral ores, the quarrying of stone, and the extraction of mineral fuels.Primary industry tends to dominate the economies of undeveloped and developing nations, but as secondary and tertiary industries are developed, its share of the economic output tends to decrease.

## Biotechnology Applications in Medicine

Biotechnology has a variety of applications in the field of medicine. Some of the biotechnology applications in medicine include the following:

### Recombinant Insulin

Insulin is required by diabetic patients to remove excess sugar from the blood. Diabetic patients have a very low level of insulin or no insulin produced by the body. Therefore, they need external insulin to control blood glucose levels.

Later it was discovered that the insulin produced by the pancreas of the pigs can be used by humans. But there were not enough pigs to provide the quantities of insulin required. This led to the cloning of the human insulin gene.

The specific gene sequence that codes for human insulin were introduced in E.coli bacteria. The gene sequence altered the genetic composition of the E.coli cells. Within 24 hours several E.coli bacteria containing the recombinant human insulin gene were produced. The recombinant human insulin was isolated from E.coli cells.

### Gene Therapy

Gene Therapy holds the most promising answer to the problem of genetic diseases. Gene therapy is used to treat genetic disorders usually by the insertion of a normal gene or correct gene for the defective or inactive gene into an individual with the help of vectors such as retrovirus, adenovirus, and herpes simplex virus.

The normal gene replaces the defective or inactive gene and carries out its functions. The therapy has the highest chances of developing a permanent cure if introduced in the earliest stages of life.

### Molecular Diagnosis

Medical diagnosis is another application of biotechnology in the health sector. Many times the pathogen concentration increases by the time the disease is diagnosed. Hence, early diagnosis and knowledge of pathophysiology are essential for an effective cure. This can be achieved with the help of techniques such as Recombinant DNA Technology, Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA), etc.

### Pharmacogenomics

Pharmacogenomics has led to the production of drugs that are best suited to an individual's genetic makeup. It can be applied in diseases such as cancer, depression, HIV, asthma, etc.

### Edible Vaccines

Vaccines are obtained by animals and cell cultures. These vaccines contain inactivated pathogens.The transgenic plants can produce antigens that can be used as edible vaccines. Antigenic proteins from several pathogens can be expressed in plants such as tomato and banana.

Transgenic sugarbeet can treat foot and mouth disease of animals, transgenic banana and tomato can cure diseases such as cholera and hepatitis B.

**Other Biotechnology Applications**

1. Fermentation is an ancient invention of biotechnology. Alcohol and bread are being produced since ages with the help of microorganisms such as yeast. In today's scenario, the cultures have been purified and genetically refined to produce high-quality food products.
2. Crop improvement by crossing the plant breeds with desired traits is another application of biotechnology in the agriculture sector.
3. Transgenic plants are genetically engineered to produce plants with desired characteristics.
4. Tissue culture is another application of biotechnology to produce a large number of plants with an explant. It also helps in increasing the number of endangered plant species.
5. It is also helpful in forensics for the identification of criminals, or in paternal disputes.

## ENZYME IMMOBILIZATION

Enzyme immobilization can be defined as the confinement of enzyme molecules onto/within a support/matrix physically or chemically or both, in such a way that it retains its full activity or most of its activity.

Enzyme immobilization is a widespread empiric technology to achieve more stable, active and reusable enzymes. The empiricism can be reduced by the application of rational design procedures employing bioinformatic tools, engineered-proteins and detailed analyses of existent data.

There are 4 methods used for enzyme immobilization, namely

(1) Non-covalent adsorption and deposition,

(2) Physical entrapment,

(3) Covalent attachment, and

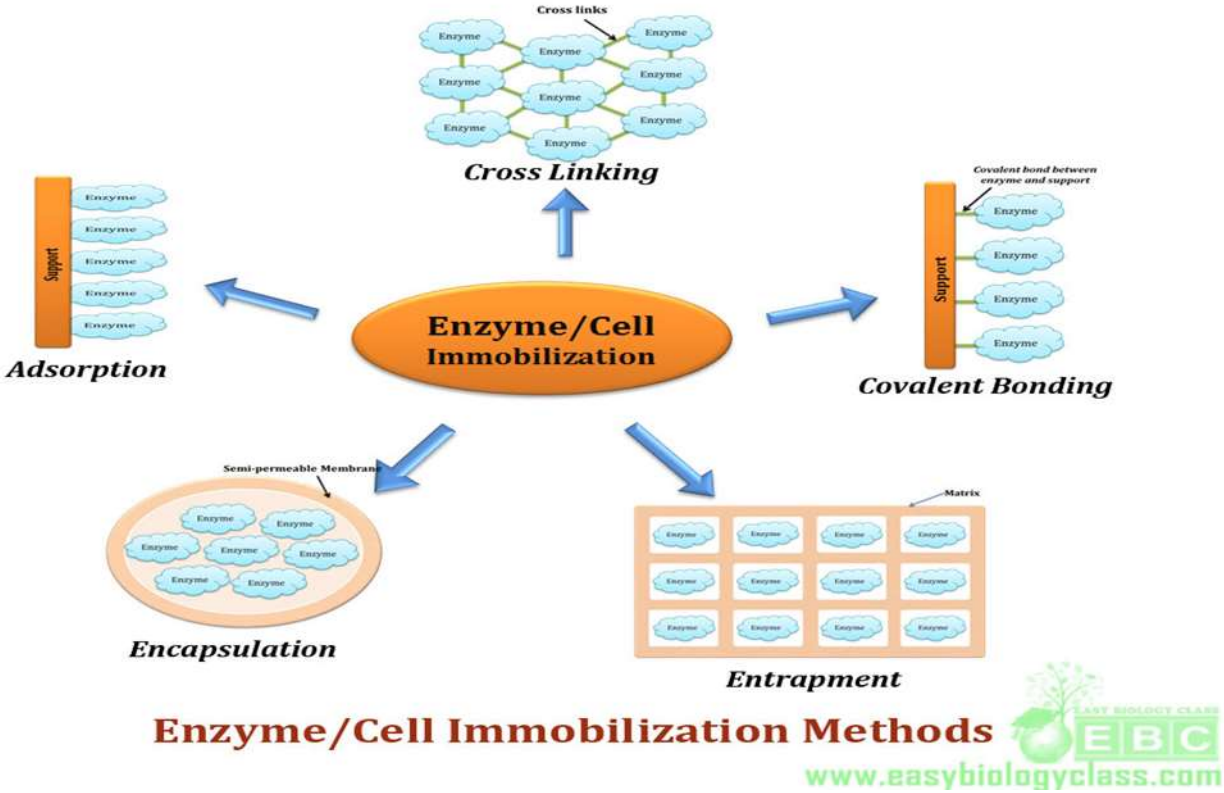
(4) Bio-conjugation: Support binding can be physical or chemical, involving weak bonds

**Adsorption**

Immobilization by adsorption is the easiest and fastest method. The adsorption is dependent on the experimental variables such as pH, nature of solvent, ionic strength, quantity of enzyme and adsorbent, the time and temperature. A close control of these variables is required owing to the relatively weak binding forces between protein and adsorbent (hydrogen bonds, van der Waals forces, hydrophobic interactions, etc.). Enzymes can be immobilized by simply mixing the enzymes with the matrix, under appropriate conditions of pH and ionic strength. Adsorption process is based on vander Waal forces, ionic and hydrogen bonding as well as hydrophobic

interactions, which are very weak forces, but in large number, impart sufficient binding strength. Adsorbed enzymes can be protected from agglomeration, proteolysis and interaction with hydrophobic interfaces. In order to prevent chemical modification and damage to enzyme, the existing surface properties of enzymes and support are need to be considered.

The adsorption through physical method generally involves multipoint protein adsorption between a single protein molecule and a number of binding sites on the immobilization surface. The main disadvantage of this method is that the enzyme is easily desorbed by factors like pH, temperature fluctuations, changes in substrate and ionic concentrations.




**Few advantages of adsorption methods are**

- Easy to carry out
- No reagent are required
- Minimum activation step involved
- Comparatively cheap method
- Less disruptive to protein than chemical methods

**Entrapment**

Entrapment is based on the occlusion of an enzyme within a constraining structure, but tight enough to release an enzyme while allowing penetration of a substrate. However, due to

diffusion limitations, such methods are often unsuitable for the immobilization of enzymes hydrolyzing macromolecular substrates.

**Methods of Entrapment**

- Inclusion in the gels: enzymes trapped in gels
- Inclusion in fibers: enzymes supported on fiber formate
- Inclusion in microcapsules: enzymes entrapped in microcapsules formed by monomer mixtures such as polyamine, calcium alginate

**Advantage of Entrapment method:**

- Fast
- Cheap (low cost matrix available)
- Mild conditions are required
- Less chance of conformational change in the enzyme

**Disadvantage of Entrapment method:**

- Leakage of enzyme
- Pore diffusion limitation
- Chance of microbial contamination

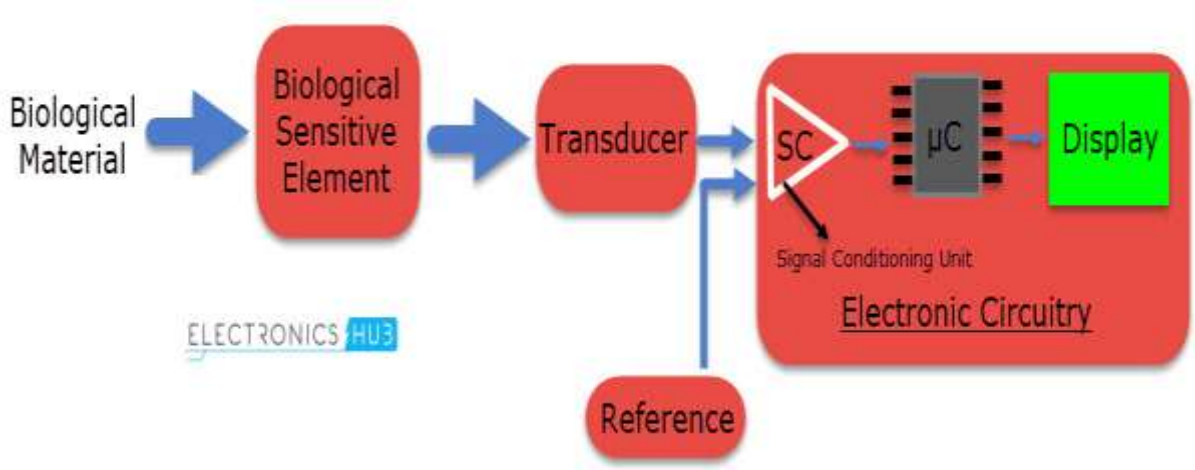
**Cross linking**

This method involves attachment of biocatalysts to each other by bi- or multifunctional reagents or ligands. In this way, very high molecular weight typically insoluble aggregates are formed. Cross-linking is a relatively simple process. It is not a preferred method of immobilization as it does not use any support matrix. So they are usually gelatinous and not particularly firm. Since it involves a bond of the covalent kind, biocatalyst immobilized in this way frequently undergoes changes in conformation with a resultant loss of activity. Still it finds good use in combination with other support dependent immobilization technologies, namely to minimize leakage of enzymes already immobilized by adsorption. It is used mostly as a means of stabalizing adsorbed enzyme and also for preventing leakage.

## BIOSENSOR

Biosensor is electronic monitoring devices that make use of an enzyme's specificity and the technique of enzyme immobilization. A biosensor is a device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction. Analyte: A substance of interest that needs detection.

Any biosensor is functionally composed of three components. The biological element, which is responsible for detecting the analyte and generating a response signal, forms the first part of the biosensor. The signal generated by the biological element is then transformed into a detectable response by the second component called as transducer, which is the most critical component in any biosensing device. The third part of the biosensor is the detector which amplifies and processes the signals before displaying it using an electronic display system.




## Different Types of Biosensors

Biosensors are classified into two groups i.e. either based on the Biological Element used in the analysis or the method of transduction implemented. As mentioned already, some of the commonly used biological elements or bio-recognition elements are DNA, enzymes, antibodies, microorganisms, tissues, cell receptors etc.

### 1) Piezoelectric Biosensors

They are a subdivision of Mass based Biosensors. Piezoelectric Biosensors are also known as Acoustic Biosensors as they are based on the principle of sound vibrations i.e. acoustics. When a mechanical force is applied on a piezoelectric biosensor, they produce an electrical signal.The biological elements are attached to the surface of the piezoelectric biosensor. The piezoelectric biosensor, which is essentially a mass to frequency converter, converts the mechanical vibrations of the sensing molecules into proportional electrical signals.

### 2) Electrochemical Biosensors

In electrochemical biosensors, the biological molecules are coated onto a probing surface. The sensing molecules are held in place with the help of non-interfering membrane. Then, the sensing molecules react appropriately to the compound to be detected and produces an electrical signal proportional to the quantity being measured.Electrochemical Biosensors can employ various types of transducers like Potentiometric, Amperometric, Impedimetric etc. converting the chemical information into a measurable electrical signal.

### 3) Optical Biosensors

Optical Fibers play an important role in Optical Biosensors. The optical fibers allow detection of the sensing elements based on the different properties of light like absorption, scattering and fluorescence. The reaction causes changes in either of the above mentioned properties as a result of the change in the refractive index of the interacting surface. For example, if the biological elements are antibodies and are bound with a metal layer, the refractive index of the medium which comes in contact with this layer will be varied. One of the main advantages of using optical biosensors is their non-electrical nature. This allows them to analyze multiple elements on a single layer just by varying the wavelength of the light.

### Applications of biosensor

1) Medicine, Clinical and Diagnostic Applications
2) Environmental Monitoring
3) Industrial Applications
4) Food Industry
5) Agriculture Industry

## PROTEIN ENGINEERING

It is the conception and production of unnatural polypeptides, often through modification of amino acid sequences that are found in nature. Synthetic protein structures and functions can now be designed entirely on a computer or produced through directed evolution in the laboratory.

There are 3 techniques of protein engineering:

1) Directed Evolution
2) Rational Design
3) 3) De novo Design

**Directed evolution:** Directed evolution (DE) is a method used in protein engineering that mimics the process of natural selection to steer proteins or nucleic acids toward a user-defined goal.[1] It consists of subjecting a gene to iterative rounds of mutagenesis (creating a library of variants), selection (expressing those variants and isolating members with the desired function) and amplification (generating a template for the next round). It can be performed in vivo (in living organisms), or in vitro (in cells or free in solution). Directed evolution is used both for protein engineering as an alternative to rationally designing modified proteins, as well as for experimental evolution studies of fundamental evolutionary principles in a controlled, laboratory environment.

The application of this theory of divergent molecular evolution to promiscuous enzymes may allow us to design enzymes with more specificity and higher activity. Many structural and biochemical analyses have identified the active or binding site residues important for functional plasticity (plasticity residues). To understand how these residues contribute to molecular evolution, and thereby formulate a design methodology, plasticity residues were probed in the active site of the promiscuous sesquiterpene synthase gamma-humulene synthase. Identified plasticity residues were systematically recombined based on a mathematical model in order to construct novel terpene synthases, each catalysing the synthesis of one or a few very different sesquiterpenes. Here we present the construction of seven specific and active synthases that use different reaction pathways to produce the specific and very different products.

**Rational design**

Rational design is a particular strategy in protein engineering, which attempts to create improved protein molecules based on the three-dimensional structure and the relationship between structure and function, which has developed over the years as part of protein science.

In rational protein design, a scientist uses detailed knowledge of the structure and function of a protein to make desired changes. In general, this has the advantage of being inexpensive and technically easy, since site-directed mutagenesis methods are well-developed. However, its major drawback is that detailed structural knowledge of a protein is often unavailable, and, even when available, it can be very difficult to predict the effects of various mutations since structural information most often provide a static picture of a protein structure.

**De novo design**

De novo design is an attractive approach for constructing designed proteins with predetermined structures and functions. The structure of a protein can be predicted by comparing the amino acid sequence to that of native 3D structure known.

De novo methods tend to require vast computational resources, and have thus only been carried

out for relatively small proteins. De novo protein structure modeling is distinguished from Template-based modeling (TBM) by the fact that no solved homologue to the protein of interest is used, making efforts to predict protein structure from amino acid sequence exceedingly difficult. Prediction of protein structure de novo for larger proteins will require better algorithms and larger computational resources such as those afforded by either powerful supercomputers (such as Blue Gene or MDGRAPE-3) or distributed computing projects.

## APPLICATIONS

**Food Industry:** Important application area of protein engineering regarding food industry is the wheat gluten proteins. Their heterologous expression and protein engineering has been studied using a variety of expression systems, such as E.coli, yeasts or cultured insect cells. Wildtype and mutant wheat gluten proteins were produced to compare them to each other for protein structure-function studies because of their availability, rapid and easy use, as well as high expression levels. Food industry makes use of a variety of food-processing enzymes, such as amylases and lipases, the properties of which are improved using recombinant DNA technology and protein engineering. The deletion of native genes encoding extracellular proteases, forexample, increased enzyme production yields of microbial hosts.

**Enzymes:** Some large groups of enzymes like Proteases, Amylases and Lipases are important for both food and detergent industries, as they have a broad range of industrial applications. Proteases are used in several applications of food industry regarding low allergenic infant formulas, milk clotting and flavors. They are also important for detergent industry for removing protein stains. The improvement of proteases for industry to have, for example, high activity at alkaline pH and low temperatures, or improved stability at high temperatures is a challenge for protein engineering. Microbial protease production is industrially suitable because of low costs, high production yields , and easy genetic manipulation. Amylases are also important for both food and detergent industries. In food industry, they are use for liquefaction and saccharification of starch, as well as in adjustment of flour and bread softness and volume in baking

**Enviromental applications:** Environmental applications of enzyme and protein engineering are also another important field. Genetic methods and strategies for designing microorganisms to eliminate environmental pollutants and included gene expression regulation to provide high catalytic activity under environmental stress conditions, such as the presence of a toxic compound, rational changes introduced in regulatory proteins that control catabolic activities, creation of new metabolic routes and combinations. Many organic pollutants such as phenols, azo dyes, organo phosphorus pesticides and polycyclic aromatic hydrocarbons can be detoxified using enzymatic oxidation.

**Petroleum biorefining** is also an important environmental application area, where new biocatalysts are required. Protein engineering, isolation and study of new extremophilic microorganisms, genetic engineering developments are all promising advances to develop new biocatalysts for petroleum refining. Petroleum biorefining applications such as fuel biodesulfurization, denitrogenation of fuels, heavy metal removal, depolymerisation of Asphaltenes. Many protein engineering strategies were identified such as improvement of hydrogen peroxide stability, increasing the redox potential to broaden the substrate range, heterologous expression and industrial production development.

**Medical Applications:** The use of protein engineering for cancer treatment studies is a major area of interest. Pretargeted radio immunotherapy has been discussed as a potential cancer treatment. By pretargeting, radiation toxicity is minimized by separating the rapidly cleared radionuclide and the long-circulating antibody. Advances in protein engineering and recombinant DNA technology were expected to increase the use of pretargeted radio-immunotherapy. The use of novel antibodies as anticancer agents is also an important field of application, where the ability of antibodies to select antigens specifically and with high affinity is exploited, and protein engineering methods are used to modify antibodies to target cancer cells for clinical applications. Recently, the term “modular protein engineering” has been introduced for emerging cancer therapies.

**Nano biotechnology:** Nano biotechnology applications of protein engineering are becoming increasingly important. The synthesis and assembly of nanotechnological systems into functional structures and devices has been difficult and limiting their potential applications for a long time.

• Biological macromolecules, such as proteins, carbohydrates and lipids are used in the synthesis of biological tissues in aqueous environments and mild physiological conditions, where this biosynthetic process is under genetic regulation. Particularly proteins are crucial elements of biological systems, based on their roles in transport, regulation of tissue formation, physical performance and biological functions. Thus, they are suitable components for controlled synthesis and assembly of nano technological systems.

# ENZYMES

## AMYLASES

An amylase is an enzyme that catalyses the hydrolysis of starch (Latin amylum) into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into sugar. The pancreas and salivary gland make amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and tri saccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria also produce amylase. Specific amylase proteins are designated by different Greek letters. All amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds.

| | **A** | **β** | **Γ** |
|---|---|---|---|
| Source | Animals, plants, microbes | Plants, microbes | Animals, microbes |
| Tissue | Saliva, pancreas | Seeds, fruits | Small intestine |
| Cleavage site | Random α-1,4 glycosidic bond | Second α-1,4 glycosidic bond | Last α-1,4 glycosidic bond |
| Reactions product | Maltose, dextrin | Maltose | Glucose |
| pH | 5.6–5.8 | 5.4–5.5 | 4.0–4.5 |
| Temperature | 68–74 °C (154–165 °F | 58–65 °C (136–149 °F) | 63–68 °C (145–155 °F) |

## USES

1. An inhibitor of alpha-amylase, called phaseolamin, has been tested as a potential diet aid.
2. When used as a food additive, amylase has E number E1100, and may be derived from pig pancreas or mold fungi.
3. Bacilliary amylase is also used in clothing and dishwasher detergents to dissolve starches from fabrics and dishes.

## CATALASES

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals) which catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS).

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains

four iron-containing heme groups that allow the enzyme to react with hydrogen peroxide. The optimum pH for human catalase is approximately 7, and has a fairly broad maximum: the rate of reaction does not change appreciably between pH 6.8 and 7.5.The pH optimum for other catalases varies between 4 and 11 depending on the species. The optimum temperature also varies by species.

Catalase has various industrial applications. In the food industry, it is used in combination with other enzymes in the preservation of foodstuffs and in the manufacture of beverages and certain food items. Commercial catalases also are used to break down hydrogen peroxide in wastewater. Catalase is an enzyme in the liver that breaks down harmful hydrogen peroxide into oxygen and water. When this reaction occurs, oxygen gas bubbles escape and create foam. Completely disinfect any surface that the raw liver touches during this activity.

**PEROXIDASES**

Peroxidase is enzymes that catalyze oxidation-reduction reaction by mechanism of free radical that transform several compounds into oxidized or polymerized products.

The prosthetic group of peroxidase is composed of a protein-bound heme, usually through a histidine residue that acts as a proximal ligand. While the exact mechanisms have yet to be determined, peroxidases are known to play a part in increasing a plant's defenses against pathogens.Many members of the Solanaceae, notably Solanummelongena (eggplant/aubergine) and Capsicum chinense (the habanero/Scotch bonnet varieties of chili peppers) use Guaiacol and the enzyme guaiacol peroxidase as a defense against bacterial parasites such as Ralstoniasolanacearum: the gene expression for this enzyme commences within minutes of bacterial attack.

Peroxidase can be used for treatment of industrial waste waters. For example, phenols, which are important pollutants, can be removed by enzyme-catalyzed polymerization using horseradish peroxidase. Thus phenols are oxidized to phenoxy radicals, which participate in reactions where polymers and oligomers are produced that are less toxic than phenols. It also can be used to convert toxic materials into less harmful substances. There are many investigations about the use of peroxidase in many manufacturing processes like adhesives, computer chips, car parts, and linings of drums and cans. Other studies have shown that peroxidases may be used successfully to polymerize anilines and phenols in organic solvent matrices. Peroxidases are sometimes used as histological markers. Cytochrome c peroxidase is used as a soluble, easily purified model for cytochrome c oxidase.

**LIPASE**

Lipase is a type of protein made by your pancreas, an organ located near your stomach. Lipase

helps your body digest fats. It's normal to have a small amount of lipase in your blood. But, a high level of lipase can mean you have pancreatitis, an inflammation of the pancreas, or anothertype of pancreas disease. Lipase is a digestive enzyme that boosts the absorption of fat in your body by breaking it down into glycerol and free fatty acids. Some studies show that supplementing with lipase may decrease feelings of fullness. Lipases are involved in diverse biological processes which range from routine metabolism of dietary triglycerides to cell signaling and inflammation. Thus, some lipase activities are confined to specific compartments within cells while others work in extracellular spaces.

In the example of lysosomal lipase, the enzyme is confined within an organelle called the lysosome. Other lipase enzymes, such as pancreatic lipases, are secreted into extracellular spaces where they serve to process dietary lipids into more simple forms that can be more easily absorbed and transported throughout the body. Fungi and bacteria may secrete lipases to facilitate nutrient absorption from the external medium (or in examples of pathogenic microbes, to promote invasion of a new host). Certain wasp and bee venoms contain phospholipases that enhance the effects of injury and inflammation delivered by a sting. As biological membranes are integral to living cells and are largely composed of phospholipids, lipases play important roles in cell biology. Malasseziaglobosa, a fungus thought to be the cause of human dandruff, uses lipase to break down sebum into oleic acid and increase skin cell production, causing dandruff.

**PROTEASE**

A protease (also called a peptidase or proteinase) is an enzyme that catalyzes (increases reaction rate or "speeds up") proteolysis, breaking down proteins into smaller polypeptides or single amino acids, and spurring the formation of new protein products. Proteases can be found in all forms of life and viruses.

Proteases are involved in many biological functions, including digestion of ingested proteins, protein catabolism (breakdown of old proteins), and cell signaling. In the absence of functional accelerants, proteolysis would be very slow, taking hundreds of years. Proteases can be found in all forms of life and viruses. They have independently evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms.

Proteases were first grouped into 84 families according to their evolutionary relationship in 1993, and classified under four catalytic types: serine, cysteine, aspartic, and metallo proteases.[6] The threonine and glutamic-acid proteases were not described until 1995 and 2004 respectively. The mechanism used to cleave a peptide bond involves making an amino acid residue that has the cysteine and threonine (proteases) or a water molecule (aspartic acid, metallo- and acid

proteases) nucleophilic so that it can attack the peptide carboxyl group. One way to make a nucleophile is by a catalytic triad, where a histidine residue is used to activate serine, cysteine, or threonine as a nucleophile. This is not an evolutionary grouping, however, as the nucleophile types have evolved convergently in different superfamilies, and some superfamilies show divergent evolution to multiple different nucleophiles.

Proteases are used in industry, medicine and as a basic biological research tool. Digestive proteases are part of many laundry detergents and are also used extensively in the bread industry in bread improver. A variety of proteases are used medically both for their native function (e.g.controlling blood clotting) or for completely artificial functions (e.g. for the targeted degradation of pathogenic proteins). Highly specific proteases such as TEV protease and thrombin are commonly used to cleave fusion proteins and affinity tags in a controlled fashion.

**PENICILLINASE**

It is enzyme that specifically breaks the β-lactam ring, thereby inactivating the antibiotic. In addition, the antibacterial spectrum of activity and pharmacological properties of the natural penicillins can be changed and improved by these chemical modifications. The addition of a β-lactamase inhibitor, such as clavulanic.

The naturally occurring penicillins, penicillin G (benzylpenicillin) and penicillin V (phenoxymethylpenicillin), are still used clinically. Because of its poor stability in acid, much of penicillin G is broken down as it passes through the stomach; as a result of this characteristic, it must be given by intramuscular injection, which limits its usefulness. Penicillin V, on the other hand, typically is given orally; it is more resistant to digestive acids than penicillin G. Some of the semisynthetic penicillins are also more acid-stable and thus may be given as oral medication. All penicillins work in the same way namely, by inhibiting the bacterial enzymes responsible for cell wall synthesis in replicating microorganisms and by activating other enzymes to break down the protective wall of the microorganism. As a result, they are effective only against microorganisms that are actively replicating and producing cell walls; they also therefore do not harm human cells (which fundamentally lack cell walls). Penicillins are used in the treatment of throat infections, meningitis, syphilis, and various other infections. The chief side effects of penicillin are hypersensitivity reactions, including skin rash, hives, swelling, and anaphylaxis, or allergic shock. The more serious reactions are uncommon. Milder symptoms may be treated with corticosteroids but usually are prevented by switching to alternative antibiotics. Anaphylactic shock, which can occur in previously sensitized individuals within seconds or minutes, may require immediate administration of epinephrine.

# GENETIC ENGINEERING

Genetic engineering is the process of using recombinant DNA (rDNA) technology to alter the genetic makeup of an organism. Genetic engineering involves the direct manipulation of one or more genes. Most often, a gene from another species is added to an organism's genome to give it a desired phenotype.

Genetic engineering, also called genetic modification or genetic manipulation, is the direct manipulation of an organism's genes using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms. New DNA is obtained by either isolating or copying the genetic material of interest using recombinant DNA methods or by artificially synthesizing the DNA. A construct is usually created and used to insert this DNA into the host organism. The first recombinant DNA molecule was made by Paul Berg in 1972 by combining DNA from the monkey virus SV40 with the lambda virus. As well as inserting genes, the process can be used to remove, or "knock out", genes. The new DNA can be inserted randomly, or targeted to a specific part of the genome. Genetic engineering is a process that alters the genetic structure of an organism by either removing or introducing DNA. Unlike traditional animal and plant breeding, which involves doing multiple crosses and then selecting for the organism with the desired phenotype, genetic engineering takes the gene directly from one organism and delivers it to the other. This is much faster, can be used to insert any genes from any organism (even ones from different domains) and prevents other undesirable genes from also being added.

Plants, animals or microorganisms that have been changed through genetic engineering are termed genetically modified organisms or GMOs. If genetic material from another species is added to the host, the resulting organism is called transgenic. If genetic material from the same species or a species that can naturally breed with the host is used the resulting organism is called cisgenic. If genetic engineering is used to remove genetic material from the target organism the resulting organism is termed a knockout organism. In Europe genetic modification is synonymous with genetic engineering while within the United States of America and Canada genetic modification can also be used to refer to more conventional breeding methods.

## Steps in Genetic Engineering

1) Identification of the gene interest
2) Isolation of the gene of interest
3) Amplifying the gene to produce many copies

4) Associating the gene with an appropriate promoter and poly A sequence and insertion into plasmids
5) Multiplying the plasmid in bacteria and recovering the cloned construct for injection;
6) Transference of the construct into the recipient tissue, usually fertilized eggs
7) Integration of gene into recipient genome
8) Expression of gene in recipient genome
9) Inheritance of gene through further generations

**Identification of the gene interest**

The most popular gene used in aquatic species is growth hormone (GH) for reasons that are obvious. GH has been widely used in terrestrial species and as the gene sequence is highly conserved; the product is readily utilized across species boundaries. It may also be noted that, at least in some cases, enhanced growth is associated with more effective utilization of food.

**Isolation of the gene of interest**

Usually the gene of interest will already be available as an element of a "library" of short sections of the total genome of the donor strain or species. If this is the case the procedure followed is to multiply the gene using the PCR reaction. If, however, the gene is to be taken from a genome not previously investigated, a more complex procedure will need to be followed. The use of the technique of the Polymerase Chain Reaction (PCR) enables the gene in both the cases noted above to be multiplied to the level of several million copies needed for the generation of the construct.

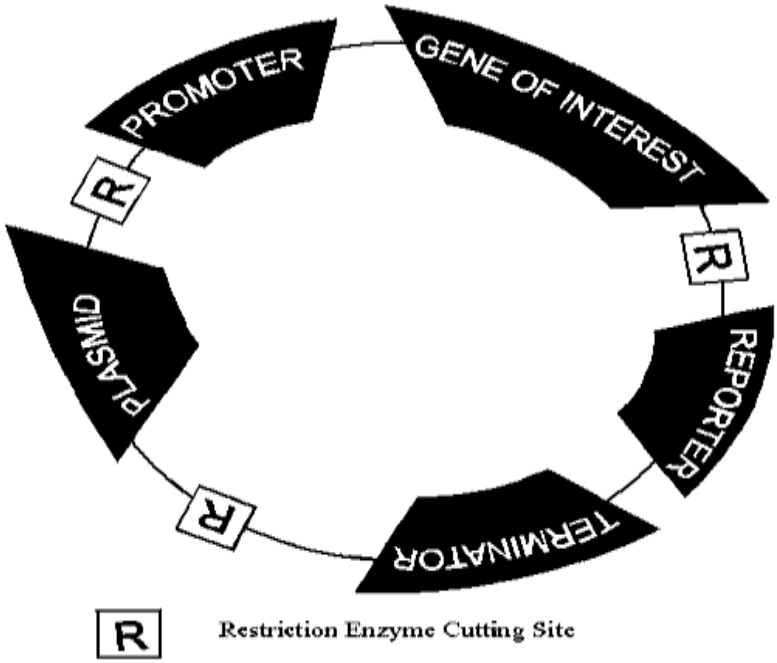
**Cloning the gene of interest**

When many copies of the target gene have been generated, the gene is placed in a "construct" Once the gene of interest has been ligated enzymatically into the construct, this whole complex is ligated into bacterial plasmids (see Figure 3), which act as "production vectors" and enable the gene to be replicated many times within the bacterial cells. The bacteria are then plated out. It is possible to tell from reporter genes (see below) whether the vector has been taken up by the bacterial cells. This usually involves some colour change in the colonies containing inserted DNA. The many times amplified DNA construct is then enzymatically cut out of the plasmids (after these have been removed from the bacterial cells) and it is ready to be used for insertion into eggs of the host species.

**The construct**

A construct is a piece of DNA which functions as the vehicle or vector carrying the target gene into the recipient organism. It has several different regions. There is a promoter region which

controls the activity of the target gene, a region where the target DNA is inserted, usually some type of reporter gene to enable one to ascertain whether the target has combined successfully with the construct and a termination sequence.




The sources of these several DNA sequences may be different species although promoter and target genes would ideally be derived from the same species.

**Techniques for inducing transgenics**

Transgenic fish have largely been produced through microinjection into fertilized eggs or early embryos. The microinjection method is suitable for relatively small numbers of organisms being manipulated whereas electroporation, sperm/liposome mediation and bombardment methods are more suitable for mass treatments. The most popular method of insertion of transgenes in aquaculture is microinjection; in 92 studies reviewed from 1985 to the present, 68 used microinjection, eleven used sperm mediated methods, six used electroporation and five used both sperm mediation and electroporation. However, the problem of mosaic expression of the transgenes is common, and this gives rise to varying proportions of transgenic genotypes in the progeny.

**Integration sites**

The factors determining sites of integration are still poorly understood though research in this direction is increasing. It is particularly important to gain greater accuracy in controlled site of integration because of the unpredictable effects of uncontrolled integration on resident genes. Caldovic and Hackett (1995) tested the ability of special sequences called transposable border

elements from other species to confer position-independent expression of transgenes or enhance integration of transgenic constructs into fish chromosomes. Early results indicate that such elements from some species do not act as enhancers and do not improve integration frequencies. However, both avian and insect border elements were found to confer position-independent expression as judged from expression of CAT genes in F1 fish. Hackett et al., (1994) showed that co-transfer of retroviral integrase protein with transgenic DNA can accelerate and enhance the rate of integration.

**Expression of gene**

The uptake and integration of a transgene does not guarantee that the gene will express itself in the new genetic environment. Tests must be carried out to determine whether there is expression and if there is expression, at what level this takes place. Clearly, in commercial aquaculture only those transgenics expressing the target gene at a sufficiently high level will be of interest.

**Inheritance of gene**

A fish which expresses the target gene at an acceptable level may not be able to transmit the gene to progeny. This is because many transgenics are mosaic individuals and unless the gonads are included in the tissues possessing the transgene the transgenic animals will not breed true. Appropriate breeding tests must, therefore, be carried out.

The high proportion of mosaic individuals is one reason why the proportions of progenies of different genotypes resulting from parents that are putatively hemizygous for a transgene do not necessarily conform to mendelian expectations. Another reason is the integration of two or more copies of the transgene at different sites in the recipient genome. Further breeding tests will be required in order to establish a pure breeding line of transgenic fish.

**Applications of Genetic Engineering**

**Application in food industry**

Genetic engineering finds application in food industry which is a result of modification of the genetic material of plants or animals. Many genetically modified (GM) whole foods or ingredients present in them available today are a result of gene modification. A number of enzymes are involved in fermentation and digestion of foods. This has led to the concept of production of recombinant enzymes from genetically modified microbes such as chymosin and lipase for cheese production, and alpha- amylase for flavor enhancement in beer industry. A mixture of enzymes called Rennet is used to coagulate milk into cheese.

**Application in pharmaceutical industry and Medicine**

By genetic engineering a variety of medical products are available today. Among these products, insulin and human growth hormone were first commercially available products obtained from

recombinant E. coli. Recombinant insulin is the result of successful genetic engineering. The initial production of insulin involved the separate synthesis of the insulin A- and B-chains in two bacterial strains. Both the insulin A and B chains genes were placed under the control of the lac promoter for inducible expression by lactose inducer. After purification of the A- and B-chains from the bacteria, the chains were then linked chemically to produce the final insulin. Recombinant-insulin is now commercially available in several forms and is involve in diabetes therapy.

**Application in Environment**

Genetic engineering is exploiting the huge potential of microorganisms, plants, animals for the restoration of the environment. Genetic engineering is actively involved in the development of microorganisms and biocatalysts for remediation of contaminated environments, and in development of eco-friendly processes such as developing recombinant strain for bio-fuel production etc.

**Application in Crop improvement through Transgenesis**

Crop plants have been the focus of genetic engineering as efforts are being made to improve the traits of plants. Transgenic plants are developed for the following reasons:

1) Gene insertion may result in improvement in the agricultural or commercial value of a plant.

2) Transgenic plants can act as a living bioreactors facilitating production of commercially important proteins or metabolites.

3) Transgenic plant helps in the understanding the function of different genes.

**Application in trait improvement of animals through transgenesis**

Genetic engineering involves the introduction of transgene into animal to improve the trait of transgenic animals. Transgenic animals thus finally express the trait of the introduced gene. Transgenic animals are also created to study the function of different genes to develop proper treatment of a disease:

1) Removal of healthy egg cells from a female host animal and fertilization in the laboratory.
2) The identification and isolation of the desired gene obtained from another species.
3) The desired genes are injected directly into the eggs and then implanted in the host female.

Transgenic animals can be a successful mean to provide an economical production of enzymes, proteins, quality and quantity improvement of meat and other animal products. The successful cloning of Dolly (a sheep) in 1997 by genetic engineering, there is continuous effort in the direction of cloning of useful livestock.

**Very Short Answer Questions (2 marks)**

1. Define Biotechnology.
2. What is Genetic Engineering?
3. Define Enzyme Immobilization.
4. What are Biosensors?
5. What is Protein Engineering?
6. What is the role of biosensors in medical field?
7. What is Gene Knock Out Technology?
8. Write examples of rDNA derived products.
9. Define Copolymerisation.
10. Write objectives of Protein Engineering.
11. Give examples of bacteria and fungi producing amylases.
12. Write Sources of Lipases.
13. What are Linkers and Adaptors?
14. Define rDNA technology.
15. Define Biological Engineering.
16. What is Pharamacogenomics?

**Short Answer Questions (5 marks)**

1. Write the major fields of biotechnology along with its applications.
2. What is role of biosensors in medical field?
3. Explain Monoclonal Antibodies.
4. Explain various methods used for immobilisation of enzymes.

**Long Answer Questions (10 MARKS)**

1. Write note on biosensors along with its advantages and applications.
2. What are the different approaches to study Protein Engineering?
3. Explain Protein Engineering with its applications.