The Academic
International Journal of Multidisciplinary Research

An Online Peer Reviewed / Refereed Journal
Volume 3 | Issue 1 | January 2025
ISSN: 2583-973X (Online)
Website: www.theacademic.in

PHARMACEUTIAL ENZYMES A BOOMING BIOMOLECULES: DEFINITION, PROPERTIES AND THERAPEUTIC USES IN MEDICINE

K. Swati

Masters In Pharmaceutical Technology; School of Pharmaceutical Technology

SOA University; Bhubaneshwar, Orissa, India

Email.Id: swatikunipalli2@gmail.com

ARTICLE DETAILS

Research Paper

Keywords:

Enzyme, Biocatalysts, Lipases, Proteases, Nucleases, Cell Culture, Fusion Tag removal, Protein Synthesis, Biosensors, Recombinant DNA and RNA synthesis.

ABSTRACT

Abstract: Enzymes are biological catalysts that speed up biochemical reactions in living organisms. For example, they have important roles in the production of sweetening agents and the modification of antibiotics, they are used in washing powders and various cleaning products, and they play a key role in analytical devices and assays that have clinical, forensic, and environmental applications. The word 'enzyme' was first used by the German physiologist Wilhelm Kühne in 1878, when he was describing the ability of yeast to produce alcohol from sugars, and it is derived from the Greek words "en" (meaning 'within') and zyme (meaning 'yeast'). Several enzymes are used in peptide synthesis, diagnostic and fragments, digestion of unwanted proteins during nucleic acid purification, proteolytic enzymes in molecular biology. Enzymes used to assist drug delivery, medical devices cleaning, diagnosis and therapies, recombinant DNA and protein technologies, cell culturing, peptide sequencing, advances in recombinant lipases, etc. Proteases in disease identification and molecular biology, some of the known marketed formulations containing Nucleases acts as a ground breaking discoveries in treating various monogenic diseases Like Cystic Fibrosis, Duchenne Muscular Dystrophy (DMD), Sickle cell anemia (SCA), Hemophilia and β-thalassemia ZFNs in pharmaceutical medicine manufacturing and their applications in Biologics and Pharmaceutical Biotechnology [3,4].

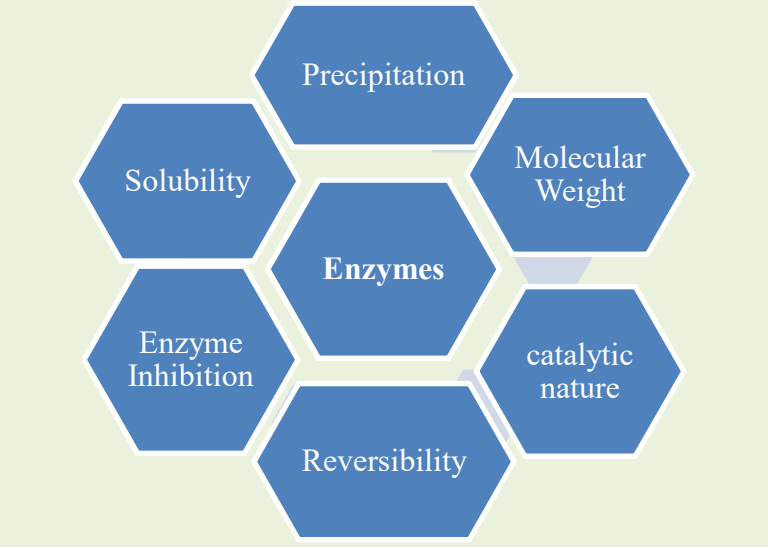
DOI: https://doi.org/10.5281/zenodo.14873099

Introduction:

As enzymes are specific biological catalysts, they must create the foremost fascinating therapeutic agents for the treatment of various diseases. They are large to be distributed merely among the body's cells. Several ways are being developed to beat this by targeting enzymes; as examples, enzymes with covalently connected external b-galactose residues are targeted at hepatocytes and enzymes covalently coupled to target-specific organism antibodies are getting used to avoid non-specific side-reactions **[1,2].**

Being typically foreign proteins to the body, they are substance and may elicit associate degree immune reaction which can cause severe and severe aversions, notably on continued use. It has tested attainable to bypass this downside, in some cases, by disguising the protein as associate degree apparently non-proteinaceous molecule by valency modification. Asparaginase, changed by valency attachment of synthetic resin glycol, has been shown to retain its anti-tumour result whereas possessing no immunogenicity. Clearly the presence of poisons, pyrogens and alternative harmful materials among a therapeutic protein preparation is completely out. Effectively, this encourages the utilization of animal enzymes, despite their high value, relative to those of microorganism origin.

Their effective time among the circulation is also solely a matter of minutes. This has tested easier than the immunologic downside to combat, by disguise victimization valency modification. Other ways have conjointly been shown to achieve success, notably those involving demurrer of the protein among artificial liposomes, hydrogels, artificial microspheres, and red somatic cell ghosts**.** However, although these methods are efficacious at extending the circulatory lifetime of the enzymes, they often cause increased immunological response and additionally may cause blood clots **[1,5].**



Diagrammatic representation of Properties of Enzymes

Properties of Enzymes:

- Enzymes can accelerate the reaction in either direction.
- All enzymes possess active sites which participate in the biochemical reactions.
- Enzymes are very unstable compounds mostly soluble in water, dilute glycerol, NaCl and dilute alcohol.
- Enzymes act actively at optimum temperature.
- All enzymes are protein in nature but all proteins may not be an enzyme.
- Enzymes lower the energy of activation of the substance molecule so the biochemical reaction can take place at normal body temperature which is 37 degrees Celsius.

Where Are Enzymes Used Today?

For thousands of years, humans have used enzymes in brewing, baking, and making cheese. While they are still employed for those purposes, the science of enzymes has evolved in leaps and bounds. This has created opportunities for new applications in many industries. Enzymes are also routinely added to detergents to help remove stains from fabrics, caked on food from dirty dishes, and patient soils from surgical instruments, including endoscopes **[4].**

Enzymes Used to Treat Disorders:

These are used in three cases:

1. To break the internal blood clots.
2. To dissolve the hardening of walls of blood vessels.
3. To dissolve the wound swelling to promote healing.

In some disorders like low blood pressure, or head or spinal injuries, there are chances of formation of blood clots. These clots lead to obstruction of blood flow to the target organ. This can be life-threatening if it is in the brain or heart which require a constant supply of oxygen and energy. The only way out then is to dissolve the clots. These clots are usually removed by dissolution by enzymes that can break them. Similarly, when there is atherosclerosis, hardening and thickening of blood vessel walls. This can lead to heart problems if untreated. The best way out at this junction is to decrease the fat intake and dissolve the formed thickenings. Enzymes like serrati peptidase and other work well.

For wound healing, the swelling formed might be painful and tend to form pus. Enzymes such as trypsin, chymotrypsin, Serrato peptidase are used to dissolve the swelling.

Enzymes Used to Assist Drug Delivery

Some medicines must be compelled to penetrate deeper tissues for higher action. For this, some enzymes are used along with drugs in intra-muscular injection forms to help proper penetration of tissues. One of such enzymes is Hyaluronidase. This is a natural human protein gift in human spermatozoa to assist spermatozoan penetrate female internal reproductive organ tissue and fertilize with ova. Here the same enzyme is manufactured by rDNA technology and administered along with drugs to enable efficient drug delivery to the target site. Indicates the disorder. This is why it is important to have enzymes in medicine. Similarly, by use of polymerase chain reaction (PCR), they help to diagnose genetic diseases in the prenatal stage for disorders like sickle cell anemia, Huntington's disease, beta-thalassemia, etc. Beginning material to medication. Also, steroidal drugs are manufactured by enzyme action on plant steroids. Immobilized enzymes are used in the manufacture of many drugs and antibiotics. This is

attainable as enzymes convert the pro-drug molecules to medication**.** Enzymes of the liver, kidney, skeletal muscle, heart, etc. leak into blood during related disorders. Measuring the amount of the corresponding protein for his or her presence in high or low levels in blood.

Table-:1. Industrial uses of Enzymes [4]

Therapeutic uses and applications of Enzymes in Medicine

Enzymes are proteins that act as catalysts, which means they speed up chemical reactions. They are found everywhere — from the bottom of the ocean to your backyard, and even inside our own bodies. Enzymes are extracted from living organisms like bacterium and moulds. They are biological catalysts capable of skyrocketing expeditiously the speed of a chemical change while not using excessive energy, and remain unchanged once the reaction is complete.

Importance Of Enzymes in Medical Device Cleaning and Industrial application:

Enzymatic detergents designed specifically for cleaning reusable medical devices have been around for decades. Enzymatic detergents are also referenced in multiple industry standards such as AAMI ST79 and the guidelines established by the FDA and the CDC. The two main enzymes used to clean medical devices today are protease and lipases. Proteases are designed to break down protein-rich sols like blood, while lipases target fatty sols like adipose tissue. Other enzymes traditionally used in this application are amylases and cellulases, which break down starch and cellulosic polymers to facilitate their removal. These target soils may be found in human waste such as faeces. Most of the enzymes are used in Various pharmaceuticals, Food processing, Textile Industry, Organic synthesis Industry and Cosmetic Industry which are tabulated in the **[Table No: 1].**

How Enzymes Work in Medical Cleaning

Most genetic diseases are results of a selected accelerator deficiency. Similarly, bound bacterium is additional morbific thanks to associate degree accelerator activity they need.

Applications of Enzymes in different diagnostic techniques:

- **Analytical tests**: Diabetics use strips of paper is made to interact with aldohexose enzyme to observe their glucose level.
- The presence of enzymes wherever they must not be gift may also facilitate to diagnose malady. For example, when the liver is diseased or damaged, enzymes leak into the bloodstream. Testing the blood for these enzymes will ensure liver injury.
- **Therapeutic accelerators**: Enzymes are typically used as medicines to interchange enzyme deficiencies in patients like the use of blood coagulation factors to treat bleeder's disease, or the opposite where proteases are accustomed to degrade fibrin; to forestall the formation of dangerous blood clots. Proteases are accustomed to clean wounds and thus accelerate the healing method.
- **Drug manufacturing**: The chemical synthesis of complicated medicine is usually troublesome, and corporations apply enzymes to perform chemical conversions. In a semi-therapeutic way, enzymes are accustomed to aid digestion, to supplement the natural amylase, lipase and protease produced by the pancreas.

➢ Many Enzymes are used as feed enzymes in different poultry farms and to detect various metabolic disorders in farm animals and to improve digestion and metabolic activity which are tabulated in **[Table No:2].**

	Enzymes	Application
Pharmaceuticals	Nitrile hydratase, Transaminase, Monoaminoxidase, Lipase, Penicillin acylase	Synthesis of intermediates for production of active pharmaceutical ingredients.
Food Processing	Trypsin, Amylase, Glucose, Papin, Pectinase, Isomerase	Conversion of starch to glucose, production of high fructose corn syrup, production of prebiotics debittering of fruit juice.
Detergents	Proteases, Lipases, amylase, cellulose	Stain removal, Fat and oil removal and colour or dye retention.
Biofuels	Lipase, Cellulase, Xylanase	Production of fatty acids, methyl esters, decomposition of lignocellulose material for bioethanol production.
Paper and Pulp	Lipase, Cellulose, Enzymes	Removal of lignin for improved bleaching, Improvement in Fiber properties
Baby Foods	Trypsin and Proteases	Trypsin and Proteases is used to predigest baby foods.
Textile Industry	Amylases, cellulases, pectinases, glucose oxidases	Removing the fuzz and microfibers from the fabric to give a smooth and shiny appearance.
Organic synthesis industry	Hydrolases, alcohol dehydrogenases, monooxygenases, fructosidases acylation, diacylation	Acylation, decylation, reduction of C-O, C=C bonds. Oxidation of alcohols and c-c sequencing
Cosmetic industry	Oxidases, peroxidases, glutathione oxidases, Papain, bromelain, glucose oxidase	Hair dying, hair waxing, peeling effect in skin care, toothpastes, and mouthwashes

Table-:2. Feed Enzyme uses and Source of Production [4]

Enzymes	Source	uses

Amylase	Aspergillus Niger, Bacillus subtilis, Trichoderma	Corn silage, Corn gluten feed, barley, grain sorghum, oat, pea, tapioca, millet, rice.
Galactosidase	Aspergillus Niger	Soyabean Meal
Cellulase	Trichoderma longibrachiatum	Corn, Barley, Wheat, wheat bran, rye, grain sorghum
Glucanase	Aspergillus Niger, Bacillus subtilis, Trichoderma	Glucose
Lipase	Aspergillus Niger	Plant and animal sources of fats and oils
Maltase	Bacillus subtilis	Maltose
Mannase	Bacillus lentus	Corn, Soyabean meal, guar meal
Pectinase	Aspergillus Niger	Corn, Wheat
Phytase	Aspergillus Niger	Corn, soyabean meal, Sunflower meal, Hominy, Tapioca, Plant byproducts
Proteases	Aspergillus Niger, Aspergillus oryzae, Bacillus subtilise	Plant and animal proteins
Xylanase	Aspergillus Niger,	Corn, Barley, Wheat, wheat bran, rye, grain sorghum

Applications of Nucleases in Biotechnology and Pharmaceutical Industry: Programmable nucleases allow defined alterations in the genome with ease-of-use, efficiency, and specificity. Their availability has led to accurate and widespread genome engineering, with multiple applications in basic research, biotechnology, and therapy **[13]**. Gene editing is a gene therapy approach that relies on designer nucleases to recognize and cut specific DNA sequences, and subsequently exploits innate cellular DNA repair pathways, namely nonhomologous end joining (NHEJ) and homology directed repair (HDR), to introduce targeted modifications in the genome. Four nuclease families have been used in this context: mega nucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regulatory interspaced short palindromic repeats associated RNA guided Cas9 (CRISPR-Cas9) nucleases **[13].** Nucleases are enzymes that break phosphodiester bonds in nucleic acids, and they play a vital role in many biological processes:

- **DNA repair**: Nucleases are involved in several DNA repair processes, including base excision repair, nucleotide excision repair, mismatch repair, and double strand break repair **[12,13].**
- **RNA processing**: Nucleases are essential for RNA processing, maturation, and RNA interference **[12,13].**
- **DNA replication**: Nucleases are an integral part of DNA replication, and are needed to remove RNA primers.
- **Recombination**: Nucleases initiate recombination, another major DNA metabolic process **[12,13].**

- **Structural alterations**: Nucleases are required for structural alterations of nucleic acids, such as topo isomerization, site-specific recombination, and RNA splicing.
- **Microbial defense**: Nucleases are an essential component of microbial defense mechanisms.
- **Programmed cell death**: Nucleases are essential for programmed cell death.
- **Function of nucleases in DNA repair [24]**
  DNA nucleases catalyze the cleavage of phosphodiester bonds. These enzymes play crucial roles in various DNA repair processes, which involve DNA replication, base excision repair, nucleotide excision repair, mismatch repair, and double strand break repair. In recent years, new nucleases involved in various DNA repair processes have been reported, including the Mus81: Mms4 (Eme1) complex, which functions during the meiotic phase and the Artemis: DNA-PK complex, which processes a V(D)J recombination intermediate. Defects of these nucleases cause genetic instability or severe immunodeficiency. Thus, structural biology on various nuclease actions is essential for the elucidation of the molecular mechanism of complex DNA repair machinery. Many nucleases utilize metal cofactors for the hydrolytic reaction **[24].** They are proposed to play any one or a combination of the following roles:
  (1) Positioning the substrate and/or the attacking nucleophile
  (2) Enhancing the nucleophilicity of the phosphate at the scissile bond
  (3) Activating the nucleophile
  (4) Neutralizing the negative charge in the transition state
  (5) facilitating the departure of the leaving group.

To examine these roles, various metals are recruited to the nuclease active sites. Depending upon the nuclease, magnesium or manganese is the most common metal used for catalysis. The magnesium ion appears to be transiently recruited to the active sites, whereas zinc and manganese are more tightly bound to the catalytic centers. The Below mentioned table Indicates the uses of nucleases in drug delivery and treating various diseases and Gene Editing strategies including the therapeutic applications in pharmaceutical industry which are represented **in [Table No: 3,4 and 5].**

Table:3. Nucleases and its delivery route [12,13]

Disease	Nuclease	Gene editing strategy	Nuclease delivery Route	Experimental Model
Cystic Fibrosis	ZFNs TALENs Cas 9	HDR mediated cDNA lock in HDR of F508 mutation using plasmid donor HDR of F508 Mutation using short DNA fragments HDR mediated cDNA knock in HDR of F508 mutation using piggyBac transposon	Plasmid transfection Plasmid transfection Plasmid Electroporation (Amaxa) Plasmid Electroporation (Lonza) Lentiviral transduction	Human bronchial and CF; Tracheal epithelia iPSCs Stem cell organoids Immortalised patient
Duchenne Muscular Dystrophy (DMD)	MGNs ZFNs Cas 9	HDR with 4.2 Kb cDNA Excision of exon 51 to restore the reading frame (applicable to 13% of patients) Exon 45 skipping by disruption of splice acceptor, NHEJ restoration of reading frame, HDR-mediated exon 44 cDNA knock-in Excision of exons 45–55 restoring the reading frame	Lentiviral transduction Plasmid electroporation (Gene Pulsar X-Cell) Plasmid electroporation (Neon Life Technologies Plasmid electroporation (Gene Pulsar X-	Immortalised Patient myoblasts

Table:3. Nucleases and its delivery route [12,13]

Disease	Nuclease	Gene editing strategy	Nuclease delivery Route	Experimental Model
		(applicable to 62% of patients) Exon 45 skipping by disruption of splice acceptor, NHEJ restoration of reading frame, HDR-mediated exon 44 Cdna.	Cell) Plasmid electroporation (Neon Life Technologies)	
Sickle cell anaemia (SCA) and β-thalassemia	ZFNs	HDR using plasmid donor HDR using IDLV/ssODN donor NHEJ-mediated disruption of BCL11A enhancers for upregulation of HbF HDR-mediated full-length cDNA knock-in HDR using piggyBac transposon	Plasmid electroporation (unknown instrument with β-thal cells, Lonza with SCA cells) Plasmid electroporation mRNA transfection (BTX device/ MaxCyte) Plasmid electroporation (Lonza)	SCA-patient iPSCs69
	Cas 9	NHEJ-mediated disruption of BCL11A enhancers for upregulation of HbF	mRNA transfection (BTX device/ MaxCyte); Plasmid electroporation (Lonza)	Mobilized human (adult) CD34+ HSCs

Table:3. Nucleases and its delivery route [12,13]

Disease	Nuclease	Gene editing strategy	Nuclease delivery Route	Experimental Model
Haemophilia	ZFN	HDR-mediated insertion of F8 and F9 cDNA within Albumin locus using AAV 8 donor	AAV-8 ZFN transduction	Humanized haemophilia B, neonatal, adult mice
	TALENs	NHEJ-mediated correction of 140 Kb inversion in F8 gene	Plasmid electroporation	Haemophilia A patient iPSCs
	Cas9	NHEJ-mediated correction of 140 Kb and 600 Kb inversions in F8 gene Cas9 protein and in vitro transcribed gRNA electroporation (Neon Life Technologies)	Cas9 protein and in vitro transcribed gRNA electroporation (Neon Life Technologies)	Haemophilia A patient iPSCs

CGD, chronic granulomatous disease; HDR, homology-directed repair; IDLV, integration-deficient lentiviral vector; iPSCs, induced pluripotent stem cells; MGNs, mega nucleases; not applicable; NHEJ, nonhomologous end joining; RS-SCID, radiosensitive severe combined immunodeficiency; ssODN, single-stranded oligonucleotide; TALENs, transcription activator-like effector nucleases; ZFNs, zinc finger nucleases.

Table:4. Therapeutic Enzymes, Treatment options and its marketed available dosage form [www.drugs.com]

Disease/Condition	Therapeutic Enzymes	Marketed Dosage Form
Gaucher's disease	Glucocerebrosidase [Cerezyme, Vprip, Taliglucerase alpha]	Injection
Hunter's syndrome	Iduronate-2-sulfatase [Elaprase]	IV Infusion Injection
Fabry's disease	α, β-galactosidase A [Replagal, Fabrazyme]	Lyophilized Powder
Hurler's syndrome	α-L-iduronidase [Aldurazyme]	Recombinant Human alpha Iduronate
Morquio syndrome type A	N-acetylgalactosamine-6-sulfate sulfatase	Injection 5 mg/ml

	[Vimizim]	
Maroteaux-Lamy syndrome	N-acetylgalactosamine-4-sulfatase [Naglazyme]	Injection 5 mg/ml
Sly syndrome	β-glucuronidase [Mepsevii]	Solution for Infusion 2mg/ml
α-Mannosidosis	Velmanase α[Lamzede]	Lyophilized Powder for Reconstitution 10 mg/ml
Batten disease	Cerliponase α [Brineura]	Injection 150 mg/ml
Pompe's disease	α-glucosidase [Myozyme]	Intravenous Infusion (50 mg)
Exocrine pancreatic insufficiency (EPI)	Pancreatic enzymes [Enzepi]	Lyophilized Powder
Phenylketonuria (PKU)	PAH and phenylalanine ammonia-lyase PAH [Palynziq]	Subcutaneous Injection
Wolman disease	Lysosomal acid lipase [Kanuma]	Intravenous Infusion 1mg/kg
Congenital sucrase-isomaltose deficiency (CSID)	Sacrosidase	Oral Solution
Hypophosphatasia	TNSALP [Strensiq]	Subcutaneous Injection
Protein C deficiency	Protein C [Ceprotin]	IU Powder
Lactose intolerance	Lactase	Capsules and Tablets
Chronic total occlusions	Collagenase *Clostridium histolyticum* (CCH)	Ointments, Tablets, Injection
Keloid disease	Collagenases and matrix metallopeptidases	Proteolytic Enzymes
Different ocular diseases treated with vitrectomy	Chondroitinase, hyaluronidase, Nattokinase and ocriplasmin	Injections and Tablets
Arthritis	Proteolytic enzymes	Tablets and Capsules
Different types of cancer	PEGylated arginine deaminase and kynurenines [Voraxaze, PEG hyaluronidase PH20]	Infection
Leukemia	L-asparaginase [Spectrila, Kidrolase, Erwinase, Oncaspar]	Injection, Infusion
Chemotherapy-induced hyperuricemia	Urate oxidase and rasburicase [Fasturtec]	IV Infusion 0.2 mg/kg
Cardiovascular disease	Nattokinase and urokinase [Streptase, Syner-Kinase, Kinclytic, Rapilsyn, Actilyse, Metalyse]	Tablets and Capsules

Burns	Collagenase *Clostridium histolyticum* (CCH) [Nexo bird]	Topical Gel
Cellulite	Collagenases	Buttock Injectable formulation
Organ injury in haemorrhagic shock	Superoxide dismutase	Intraperitoneal injection
Parkinson's	Nanozyme (PtCu nanoalloys)	Tablets and Capsules
Celiac disease	Gluten-degrading enzymes	Injections
Microbial infections	Matrix-degrading enzymes (polysaccharide-degrading enzymes, nucleases, and proteases)	Tablets, Capsules, and Injection
Inflammation	Proteolytic enzymes (trypsin or serratiopeptidase)	Tablets, Capsules, Injections

Applications of Proteolytic Enzymes in Field of molecular biology and Recombinant DNA Technology:

Proteolytic enzymes are capable of hydrolyzing peptide bonds and referred to as peptidases, Proteases or Proteinases. Depending on their site of enzyme action the proteases can also be subdivided into exopeptidases. Exopeptidases catalyses the hydrolysis of the peptide bonds near the N terminal ends of the substrate, Aminopeptidases can liberate single amino acids, Dipeptides, or tripeptides from the N terminal end of their substrates.

A) **Nucleic acid Isolation [10]**: The first step of nucleic acid isolation protocol is the Lysis of the Biological material containing the DNA and RNA of interest [3]. Before the purification and Concentration of Nucleic acids the contaminating proteins and the other macromolecules must be removed from the sample. Undamaged nucleic acid can be isolated when the degradation of the DNA and RNA present in the sample is avoided by the Inhibition of and removal of DNA and

RNA.The nucleases can be inhibited by the addition of chelators which binds the ions essential for their action. Proteases K is the most widely used proteases which leads to the higher yield of the DNA and RNA to be isolated [5].

B) **Cell Culturing [9]:** - Cells isolated from the tissue can be cultured separately from the organism in Cell Culture flasks using appropriate growth medium. Adherent Cells grown in a cell culture flask are attached to the surface by Protein bridges which must be disrupted during passaging.
The cells can be released from the cells flask surface mechanically using a cell scrapper or can be detached by a protease's treatment using trypsin solution **[15].** Trypsinization means the process used for the treatment or Detachment of adherent cells using trypsin solution to digest the adhesion molecules by which the cells are attached to the surface of the culture flask. Trypsin solutions generally contain EDTA to reduce the concentration of the metal ions that might inhibit trypsin.

C) **Fusion Tag Removal [8]:** The Protein Produced by Recombinant DNA technology is typically Linked a Fusion tag means the fusion of ana additional proteins or peptides to the recombinant Protein. These Tags are extensively used Basic research to high throughput Structural biology owing to the several advantages they provide in expression of proteins. Commonly used affinity tags and the fusion protein Partners are the hex histidine tag, Flag tag, maltose binding protein, Glutathione S, Transferase (GST), Thioredoxin, Small ubiquitin like modifiers (SUMO), Ubiquitin (Ub) and fluorescent protein.

D) **Proteomic Applications: -** Proteomics can be defined as the systematic determination of protein sequence, Quantity, Modification state, Interaction Partners, activity, Subcellular Localization Structure in each cell type at a particular time. In Proteomic analysis Mass spectroscopy (MS) is widely used proteolytic enzyme for protein digestion **[3]**. Due to its high specificity, it is easy to predict the cleavage sites and to compare the results of experimental enzymatic and Theoretical in SILICO digestion. Trypsin is the most widely used

E) **Enzyme Production and its source [11]:** Recent advancement and the increasing global demand of commercial enzymes lead to discovery of several scale up technologies and introduced different varieties of enzymes to global market in production of beverages, animal, and poultry farm feed us, proteases and lipases in industry and biotechnological applications. A few examples are tabulated below to understand the enzyme, source, and its usage. Proteases are classified on the basis of catalytic mechanisms which are tabulated in **[Table No:6].**

Table: 5. Substrate Specificity of Proteolytic Enzymes Used in Molecular Biology Research [3]

Enzyme	Main source	Cleavage site
Serine proteases		
Trypsin	Bovine	Arg or Lys nonspecific
Chymotrypsin	Bovine	-Trp (or Phe, Leu, Tyr)
Enterokinase bovine	Bovine	Asp-Asp-Asp-Lys↓nonspecific
Endoproteinase Arg C	microbial	Arg↓nonspecific
Endoproteinase Glu C	microbial	Glu (or Asp) ↓nonspecific
Endoproteinase Lys C	microbial	Lys nonspecific

Elastase	Porcine	Ala (Gly or val) ↓nonspecific
Subtilisin	Microbial	Trp (Tyr, Phe, Leu) Nonspecific
Proteinase K	Fungal	Aromatic, Aliphatic, Hydrophobic nonspecific
Thrombin	Bovine	Specific for Leu-val-pro-Arg- Gly-Ser
Factor Xa	Bovine	Specific for Leu-val-pro-arg-gly-ser
WNV Protease	E. coli	Lys-Arg -Gly-Ser
Cysteine Proteases		
Bromelain	Plant	Nonspecific
Papain	Plant	-Arg Nonspecific
Ficin	Plant	Nonspecific
Rhinovirus	E. coli	-Nonspecific; Gly-Pro dipeptide after the scissile bond
TEV Proteases	E. coli	Specific for Gln-Asn-Leu-Phe-Gln-Gly
TVMV Proteases	E. coli	Specific For Glu-Thr-Val-Arg-Phe-Gln-Ser
Metalloproteases		
Endopeptidases Asp-N	Microbial	Nonspecific -Asp
Thermolysin	Microbial	-Leu, Leu (Phe, Val, Met, Ala)
Collagenase	Microbial	Pro Neutral -Gly-Pro
Dispase	Microbial	Nonspecific non polar
Aspartic Proteases		
Pepsin	Porcine	-Phe (or Tyr, Leu, Trp) ↓Trp (or Phe, Tyr, Leu)-
Cathepsin	Bovine	-Phe (or Leu) ↓nonspecific (not Val, Ala)-

Application of Lipases in Pharma and Biotechnological Research [15,16]: Lipid constitutes a large part of the earth's biomass and lipolytic enzymes play an important role in the turnover of these water-insoluble compounds. Lipolytic enzymes are involved in the breakdown and mobilization of lipids within the cells of individual organisms as well as in the transfer of lipids from one organism to another (Beisson et al., 2000). Lipases are one of the important groups of biocatalysts used in biotechnological applications (Benjamin and Pandey, 1998). Lipases have been isolated from many species of plants, animals, bacteria, fungi, and yeast. Lipases extracted from microorganisms are used in various industries such as dairy, food, detergents, textile, pharmaceutical, cosmetic and biodiesel industries. It is also used for synthesis of fine chemicals, agrochemicals, and new polymeric materials **[6].**

Microbial Sources of Lipases and Its Purification Methodologies: Sources for microbial lipases Microbial lipases found universal in nature and are commercially substantial due to the low manufacturing cost superior stability and more availability than animal and plant lipases. Naturally or recombinant microbial lipases are generally used in diverse bioengineering applications. The novel purification machineries such as the (i) membrane separation procedures, (ii) immunopurification, (iii) hydrophobic interaction chromatography using epoxy activated spacer arm as a ligand and polyethylene glycol restrained on Sepharose, (iv) polyvinyl alcohol polymers as column chromatography stationary phases, and (v) aqueous two-phase systems are frequently engaged after these pre-purification steps **[7].** The enzyme recovery and fold purification outcomes are found acceptable using of hydrophobic

interaction chromatography. An acid resilient lipase has been filtered from crude profitable arrangements by size exclusion on Bio-gel-p-100 and ion exchange on Mono-Q., From A. Niger fungi. Using the chromatography on hydroxyapatite, octyl-Sepharose and sephacryl S-200 the lipase was purified to homogeneity from R. japonicus NR400 **[Table:7**]

Nanotechnology in enzyme biosensors:

Definition of Biosensors: "Biosensors" **[16,17]** are devices that can detect the presence of specific analyte via their interaction with biological material such as enzymes, antibodies, and genomes. These biological materials act as elements of recognition and can attach to a physiochemical detector that will produce a measurable signal and whose intensity will differ depending on the class of analyte. Biosensors must provide fast, specific, and sensitive transduction of biochemical signals." **[Table:8]** In biosensor planning a significant role played by the nanotechnology, less than 100 nm smaller dimensions which involves in the study of manipulation, creation, and use of materials, devices. Incorporating enzymes with nanomaterials the electrochemical biosensors are new ingredients with synergistic possessions initiated from the apparatuses of the hybrid combinations **[18].** A new generation of bioelectronics devices with high sensitivity and stability has an excellent scenario based on nanotechnology biosensors. To achieve direct wiring of enzymes to electrode surface using nanoscale materials this promotes electrochemical reaction, commanding nano barcode for biomaterials, and signal amplifying of biorecognition event. Carbon nanotubes (CNT) and gold are regularly used nanomaterial for enzyme biosensors **[19].** Gold showed more catalytic ability for several organic reactions. So, to catalyse biochemical reactions to design biosensors metal nanoparticles have been used. The central idea is to use nanoscale materials for the diagnosis, monitoring, control, prevention, and treatment of diseases. Several enzymatic biosensors based on nanomaterials have been employed across different industries.

Table:6 microbial and fungal source of Lipases [8]	
Microbial source Lipase	**Industrial uses**
Fungal Source	
Fusarium solani NFCCL 4084	Halophilic lipase for biodiesel production
Yarrowia lipolytica	Degrades very efficiently hydrophobic and unusual substrates such as n-alkanes, oils, fats, and fatty acids as low-cost carbon sources
Aspergillus oryzae	Saturated fatty acids synthesized, faster cheese ripening, favour customized cheese
Rhizomucor javanicus	Non-hydrogenated solid fats
Rhizomucor miehei	Cocoa-butter equivalents
Geotrichum candidum and C. antarctica	Through biocatalytic processes preparation of chiral intermediates which synthesized the pharmaceutical compounds related to the elimination of bad cholesterol for the treatment of the Alzheimer's disease
Candida antarctica	Oils and fats enriched, removal of size lubricants, denim finishing
Candida rugosa	Candida rugosa Human Milk fat substitute
Candida lipolytica	Cheese ripening, Fatty acid production
Penicillium camembertii	Production of glycerol glycolipids, Synthesis of saturated triacyl glycerides
Trichoderma lanuginosus	Produced a lipase containing detergent 'LipoPrime®'
Penicillium roquefortii	Production of characteristic favour of blue cheese in dairy products
Aspergillus Niger	Faster cheese ripening, favour customized cheese, Dough stability and conditioning
Macerozyme guilliermondii	Promising feed lipase using cheese whey
A. Niger	GZUF36 Potential of the enzyme in the synthesis of functional oils
Aspergillus favus	Fat stain elimination; Synthesis of pharmaceuticals, polymers, biodiesels, biosurfactants
Candida antarctica	Pitch control in paper and pulp industry, Polycondensation, ring opening polymerization of lactones
Rhizomucor meihei	As a biocatalyst in personal care products such as skin and sun-tan creams, bath oils etc
Candida tropicalis, Aspergillus oryzae	Degradation of crude oil hydrocarbons
Penicillium abandum	Use for docosahexaenoic acid enrichment of tuna oil
Rhizopus nodosus	Leather processing and dehairing and fat removal

Table:6 microbial and fungal source of Lipases [8]	
Microbial source Lipase	**Industrial uses**
Candida rugosa	Activated sludge treatment, aerobic waste treatment
P. chrysogenum	Food industry waste treatment
Rhizomucor meihei	Surfactants for baking industry, Dairy products, Noodles
Candida rugosa	Candida rugosa Activated sludge treatment, aerobic waste treatment
Bacterial species	
Achromobactin sp.	Treatment of oily wastewater
Pseudomonas Mendocino	Dishwashing/laundry Removal of fat strain
Acinetobacter radioresistant;	Bacillus sp. FH5 Used in detergent industry
Staphylococcus pasteurize	Using in oil degradation
Achromobactin sp. HEGN 014, Virg bacillus pantothenticus	Treatment of oily wastewater
pseudomonas sp.	Food processing and oil manufacture
Natronococcus sp.	Application in biocatalysis
P. alcaligenes	Alkaline lipases, able to removing fatty stains when used in a washing machine
Pseudomonas plantarii	Solvay Enzyme Products, Applicable for is a non-ionic and/or anionic detergent formulation
Chromobacterium viscosum	Detergent formulations containing alkaline lipase used in laundry
Bacillus thermocatenulatus	Used in medical industry

Table:7. Electrochemical assays at lipase-based Biosensor [12, 17,18]			
Source of used lipase	**Analyte**	**Principle of lipase use in assay**	**Detection limit**
Candida rugosa (Fungi)	Methyl parathion (p-nitrophenyl pesticides)	On a glass pH electrode lipase was mobilized and transformed which reduced the pH; methyl-paraoxon inhibit reaction	93 µmol
Burkholderia cepacia Lipase (Bacterium	Lipase (Bacterium) Methyl parathion, (p-nitrophenyl)	Lipase was immobilized on zeolitic nanoparticles and then into chitosan on a glassy carbon electrode, pesticides like methyl parathion were hydrolysed to p-nitrophenyl that was electrochemically oxidized	0.1–38 µM/
Candida rugosa (Fungi)	Diazinon	Lipase converted diazinon to diethyl phosphorothioic acid and 2-isopropyl4-methyl-6- hydroxy pyrimidine. which caused a change in the impedance of the medium	10 nmol/l (fungal lipase)
Candida rugosa (Fungi)	Clofenvinphos, Malathion	Lipase converted p- nitrophenyl acetate to p- nitrophenol and acetic acid, p-nitrophenol was oxidized and a current at 0.024 V was recorded, analysed inhibited lipase, and stopped the reaction	84.5 µmol/l for clofenvinphos and 282 µmol/l for malathion
Candida antarctica, Yarrowia lipolytica and fungus	Lipase itself	p-nitrophenyl butyrate hydrolysis to butyric acid and p-nitrophenol, coloration caused by p-nitrophenol was measured	0.05 U/m

Future Challenges and Scope of Research:

Fundamental research in enzymology have remarkably developed during the past few years. Immobilization enzyme and Cell technology might be a kind of breakthrough innovation in this field. From the industrial point of view enzymology is finding its new ways in developing new enzyme related technologies **[25, 26]:**

a) Rational redesigning of the enzyme using site specific mutagenesis and 3D protein structures and new simplified biocatalyst.
b) Mining of novel genes encoding the target enzyme or proteins.
c) Tools for food production and processing in large scale.
d) Pharmaceutical uses in developing new medicines through recombinant DNA technology and vector technology.
e) Analytical and measurement tools.
f) Methods for screening new physiologically active substances.
g) Methods for creation of new sources of energy and raw materials.
h) The increase demand of animal and human feed enzymes.
i) Therapeutic treatment options for treating various genetic and rare diseases.
j) Enzyme extraction from marine, bacterial and fungal source

Conclusions: Enzymatic Processes have replaced the Conventional Chemical methodologies for the synthesis of any type of Pharmaceutical active Ingredients such as the Semisynthetic antibiotics, Active enantiomers of drugs through kinetic Resolution **[23,24].** Several enzymes like Nucleases, Proteases and Lipolytic Enzymes increased the scope of Biotechnological and Pharmaceutical technology industry towards the most promising and conventional ways of sourcing or extraction of varied enzymes and substrate specificity Cleavage sites to simplify the DNA, RNA, amino acid, and protein Synthesis. In past few years due to the large-scale requirement of different Feed Enzymes in Farm, Poultry industry, Detergent manufacturing process, uses in various disease conditions and its therapeutic Application, DNA, RNA Replication, Gene Editing, Recombination DNA Technology and Extraction of Enzymes from different microbial source has emerged one of the promising techniques. In medicine World where the proper conservation of environment and natural resources are very important for outcomes. The pharmaceutical enzyme applications will see the fastest growth as growing per capita income in the developing regions lead to greater access to healthcare. A great number of new genomics, vectors, genetically modified stains, genetic toolbox and Immobilization techniques and chromatographic matrices are currently available and there will be an increasing trend in the coming years. The Commercialization and Scale up technologies are set to make the simplified version of the synthesis of enzymes.

REFERENCES:

1.Gautam Kumar, Meghawanshi, Navpreet Kaur, Swati Verma, Narendra Kumar Dabi, Abhishek Vasistha, PD Charan, Praveen, HS Bandari, N Bhojak, Rajendra Kumar; "Enzymes for Pharmaceutical and Therapeutic Applications."8 May 2020; Wiley online library.com.

2.Shuang Li, Xiaofeng yang, Shuai yang, Muzi Zhu, Xiao wang; "Technology prospecting on enzymes "application, marketing, and engineering; volume No: 2; issue 3, September 2012, school of Bioscience and Bio engineering, China.

3.Janas Andras Motyan, Ference Toth and Jozsefi Tozer, "Research applications of proteolytic enzymes in molecular Biology." Biomolecules 2013, 3, 923 to 942; 6 November 2013.

4.Jordan Chapman, Ahmed E. Ismail and Cerasela Zoica Dinu**; "**Industrial Applications of Enzymes: Recent Advances, Techniques, and Outlooks;" Catalysts 2018,1 to 28, 238.

5. Katya Kamenova, Anna Prancheva, Stiliyana Stoyanovak, Lyubomira Radeva, Ivanka Pencheva-El Tibi, Krassimira Yoncheva, Martin A. Ravutsov, Maya K. Marinova, Svilen P. Simeonov, Simona Mitova, Rumyana Eneva, Maya M. Zaharieva, Hristo Najdenski and Petar D. Petrov; "Functional Hydrogels for Delivery of the Proteolytic Enzyme Serratiopeptidas"; Gels 2024, 10, 156.

6.Prem Chandra, Enespa, Ranjan Singh and Pankaj Kumar Arora; "Microbial lipases and their industrial applications: a comprehensive review; Chandra et al. Microbe Cell Fact (2020) 19:169.

7.FOGH, J. (Ed.) (1975). Human Tumour Cell In Vitro. New York: Plenum Press HOORN, B. and TYRRELL, D. A. J. (1969).

8. Fabiano Jares Contesini, Marcelo Gomes Davanço, Gustavo Pagotto Borin 3, Katherina Garcia Vanegas, João Pedro Gonçalves Cirino Ricardo Rodrigues de Melo, Uffe Hasbro Mortensen, Kristiina Hildén, Daniel Rossi Campos and Patricia de Oliveira Carvalho; Advances in Recombinant Lipases: Production, Engineering, Immobilization and Application in the Pharmaceutical Industry; Received: 27 July 2020; Accepted: 3 September 2020; Published: 9 September 2020.

9.Organ cultures in virology. Progress in Medical Virology, 11, 408 KEAY, L. (1978).

10. The cultivation of animal cells and production of viruses in serum-free systems. Methods in Cell biology, 20, 169.

11.NILSSON and PONTEN, J. (1975). Classification and biological nature of established human hematopoietic cell lines. International Journal of Cancer, 15, 321.

12. Abhijit Ray HOD, Department of Biotechnology, Raipur Institute of Technology, Raipur; "Application of Lipase in Industry;" Asian J. Pharm. Tech. 2012; Vol. 2: Issue 2, Pg 33-37.

13. Tatsuya Nishino1 and Kosuke Morikawa; Structure and function of nucleases in DNA repair: shape, grip, and blade of the DNA scissors; Department of Structural Biology, Biomolecular Engineering Research Institute (BERI), 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan.

14. Koutinas AA, Wang R, Webb C. Restructuring upstream bioprocessing: technological and economic aspects for production of a generic microbial feedstock from wheat. Biotechnology Boeing. 2004;85(5):524–38. 132. Chen P.

15. M, Chen Y, Wang L, Li Y, Chen Q, Deng S. Review of biological and engineering aspects of algae to fuels approach. Int J Agric Bio Eng. 2010;2(4):1–30.

16. Li H, Liu S, Dai Z, Bao J, Yang X. Applications of nanomaterials in electrochemical enzyme biosensors. Sensor. 2009;9(11):8547–61.

17. Holzinger M, Le Gof A, Cornier S. Nanomaterials for biosensing applications: a review. Front Chem. 2014; 2:63.
18. Hou S, Zhang A, Su M. Nanomaterials for bio sensing applications. Nanomaterial.2016; 6(4):58.
19. Abu-Salah KM, Alrokyan SA, Khan MN, Ansari AA. Nanomaterials as analytical tools for Geno sensors. Sensors. 2010;10(1):963–93.
20. Zhang Y, Chen X. Nanotechnology, and nanomaterial-based no-wash electrochemical biosensors: from design to application. Nanoscale.2019;11(41):19105–18.
21. Tatsuya Nishino and Kosuke Morikawa; "Structure and function of nucleases in DNA repair: shape, grip and blade of the DNA scissors;" Oncogene (2002) 21, 9022 – 9032.
22. Miguel de la Fuente, Laura Lombardino, Alfonso Gómez-González, Cristina Solari, Iñigo Angulo-Barturen, Arantxa Acera, Elena Vecino, Egoitz Astigarraga and Gabriel Barreda-Gómez; "Enzyme Therapy: Current Challenges and Future Perspectives; Int. J. Mol. Sci. 2021, 22, 9181. Received: 31 July 2021 Accepted: 23 August 2021 Published: 25 August 2021.
23. Peter K. Robinson College of Science and Technology; "Enzymes: principles and biotechnological applications;" Essays Biochem. (2015) 59, 1–41.
24. S. H. G. Allen,2 r. W. Kellermeyer, L. Stjernholm, and Harland g. Wood Department of Biochemistry, Western Reserve University, School of Medicine, Cleveland, Ohio. "Purification and properties of enzymes involved in the propionic acid fermentation;" Journal of Bacteriology Vol. 87, No. 1, pp. 171-187 January, 1964.
25. Francisco T. T Cavalcante, Italo R.de A. Falcao, Jose E da Souza, Thales G. Rocha, Isamayra G. de Souza, Antonio L.G Cavalcante, Andre L.B de Oliveira, Jose C.S dos Santos; "Designing of nanomaterials based on enzymatic Biosensors: Synthesis, Properties and Applications." Electrochem 2021, 2(1), 149-184.Mehak Baweja, Lata Nain, Yutaka Kawarabayasi and Pratyush Shukla, "Current technologies improvements in Enzymes towards their biotechnological applications." Frontiers in Microbiology; 26 April 2016; published 16 June 2016.
26. www.google.com
27. www.drugs.com
28. Kumar D; Bhalla, T.C Microbial Proteases in peptide synthesis: Approaches and applications. Polymicrobial. Biotechnol. 2005,68,726-736.
29. Morihara, K. Using Proteases in peptide synthesis. Trends Biotechnol, 1987,5,164-170.
30. Bhalla, T.C; Kumar, D; Gajju, H; Aggarwal, H.O. Thermophilic bacterial Proteases. J. Punjab.Acad. Sci 1999, 1, 77-91.