

An Apodictic Review on Recent Approaches in Enzyme Technology

Chithra Ashok ¹ , Dinesh Palanimuthu ¹ , Sharmila Devi Velusamy Selvadurai ¹ , Rudha Varshini Ammasai ¹ , Preethi Pethappampatti Senthilkumar ¹ , Rajaseetharama Sekar ^{1,*} 

¹ Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode, Tamil Nadu, India-638401

* Correspondence: rajaseetharama@bitsathy.ac.in (R.S.);

Scopus Author ID 57222124942

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Abstract: Enzymes are the most powerful biochemical moieties, predominantly the working tools in all living systems. Many studies have revealed the usage of various enzymes even in the pre-historical periods. Enzymes are known to be the extremely active biocatalyst that is widely involved in many metabolisms. Living systems explore these biomolecules for their metabolism and are exhaustively explored for various industrial and clinical applications. Due to the increasing need for enzyme-based products, various recent research focuses on exploring distinct enzymes & enzyme sources with relatively enhanced characteristics. The elegant motive of this review is to enable the readers and enzyme researchers to compend the basics of enzymes, explore the enormous recent clinical & industrial applications of enzymes like amylase, cellulase, protease, lipase, and esterase. And also, the review highly emphasizes the various enzyme source and their enriched properties like enzyme activity, annotated by recent research works carried out by various research teams across the globe. The review also accentuates the recent advancements in production technologies and high throughput activity prediction assays for the above-mentioned industrially important enzymes.

Keywords: enzymes; enzyme sources; therapeutical enzymes; industrial enzymes; high throughput enzyme assays.

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1. Introduction

Enzymes are the most basic biocatalyst for the catalysis reaction of the substrate. It is a globular protein of high molecular weight with a long linear chain of amino acids like other proteins and having specific properties that depend on the structure. They are made up of a three-dimensional complex of polypeptide chains [1]. Enzymes are available in a wide source such as plants, animal tissues, and several microorganisms, and they are used in the textile and manufacturing industries as an alternative to toxic chemicals for many processing procedures [2]. Nowadays, they are rapidly important in some sectors like pharma, green chemistry, and sustainable technology. The global enzyme market in 2020 was around USD 7.5 billion, and the annual growth rate between 2016 to 2021 is 8.2% [3,4]. The global enzyme market is planned to show high profitable growth of enzymes in the field of detergent, pharmaceutical, and even in the chemical sectors. Enzymes are more sensitive to various entities; they get inactive in high temperatures, acids, radiation and are also easily denatured by some biological factors [5]. Enzymes are powerful biocatalysts. Lowering the activation energy can increase

the reaction rate in lower concentration and carry out the reaction without consuming and undergoing any changes [6]. They are specific in their function and reaction, with that different enzyme performs various mechanism including covalent-catalysis, acid-base, electrostatic [7,8]. The biochemical reaction carried out by plants, microbes, and animals is the result directly produced by the enzyme catalysis. Mostly, the biochemistry background is indirectly or directly related to enzyme history. The basic building blocks of the living system have the capability to use biocatalyst known as enzyme effectively [9]. Enzymes have vast applications in the pharmaceutical industry to treat various ailments like cancer, etc. They are used to reduce tumor tissue inflammation, infectious pathogens prevention from tumor tissues, and so forth [10]. Not only in the pharma sector, but they also have more applications in other fields like food processing, textile processing, paper industry, etc. The enzymes reviewed in this study are amylase, cellulase, protease, lipase, and esterase. These enzymes have more therapeutic potential [11-13]. Amylase has been considered important among the other enzymes, and the amylase produced from pineapple stem is about 34.4% and from the lotus stem is about 20-30% [14]. Amylase is produced by animals, fungi, molds, bacteria, and plants but bacteria and fungi are the most important sources for amylase production in the industries [15]. It is used to degrade the starch into oligosaccharides; also, it will improve the yield of the product. The raw starch hydrolysis by amylase was a major breakthrough in the starch processing industry, reducing the cost of starch-based products [16]. It can be used in fruit drinks, textile, detergent, and alcohol & beverages industries [17]. It is also used as functional biomaterials such as adsorbent, cosmetic, carrier, agriculture, or structure-directing agent in food, pharmaceutical, paper, and tissue engineering [18]. Cellulase is an essential industrial enzyme in the global enzyme market [19]. In oil & petroleum, food processing fields, the cellulase enzyme plays a major role. The most abundant biomass (lignocellulose) acts as a renewable source for biofuels & other value-added products. And efficiently hydrolyzed by the microbial cellulases. So, it was the most important research field for researchers and industrialists [20,21]. The protease enzymes are also known as proteolytic, peptidase, or proteinase, which are present in bacteria, plants, animals, some kind of algae, and also viruses. Approximately 40% of total worldwide sales in the industrial enzyme are protease. Protease enzymes are available from many organisms, even though some are only considered for commercial uses [22]. Protease can degrade the proteins which are mainly used in the animal feed and leather industry [6]. Lipase plays an important role in dairy product fermentation since ancient days [23]. Lipid metabolism in the way of in-situ and the multifaceted ex-situ application in industries were carried out by lipase. Lipase can degrade oil and fatty acids. It is mostly used in the detergent and dairy industries. Due to their interesting properties, lipase plays a role in different modern areas, for example, agro-substance, paper assembling, cosmetics, etc. [24]. Esterase can degrade the ester into alcohol and acid, which is also considered an important enzyme in biotechnology. Due to its capability, esterase can survive in the environment, and it is distributed widely in animals, microorganisms, and plants. In organic solvents, they can be active and stable. It is one of the tremendous properties of the esterase [25,26]. It plays an important role in industries like oleochemical, dairy, and biodiesel. Also, thermostable esterase are suited for industries due to their stabilization in high temperature [27,28]. Hence, the various enzyme plays several commercially and clinically significant role in this industrial era. This review completely focuses on highlighting recent diverse application, source, bioprocess approaches, and high throughput assay techniques.

2. Materials and Methods

This review gives the cumulative idea about the basis of the enzyme, sources of enzyme, application, high throughput techniques used for enzyme assays, and advanced bioprocess approaches. Various recent scientific researches and reviews have supplemented this article. This review is comprised of 175 references covering the above-mentioned topics. Among these references, the most cited papers are recent published articles (around the years of 2018-2021, about 61.71%). This is to bring out the enzyme importance in recent years and its novel application around the multidisciplinary fields. The second most cited reference belongs between 2011-2017, around 26.29%. After that, 10.29% of sources were cited between 2001 and 2010, and the remaining 1.71% is below 2000. The details are graphically explained in Figure 1.

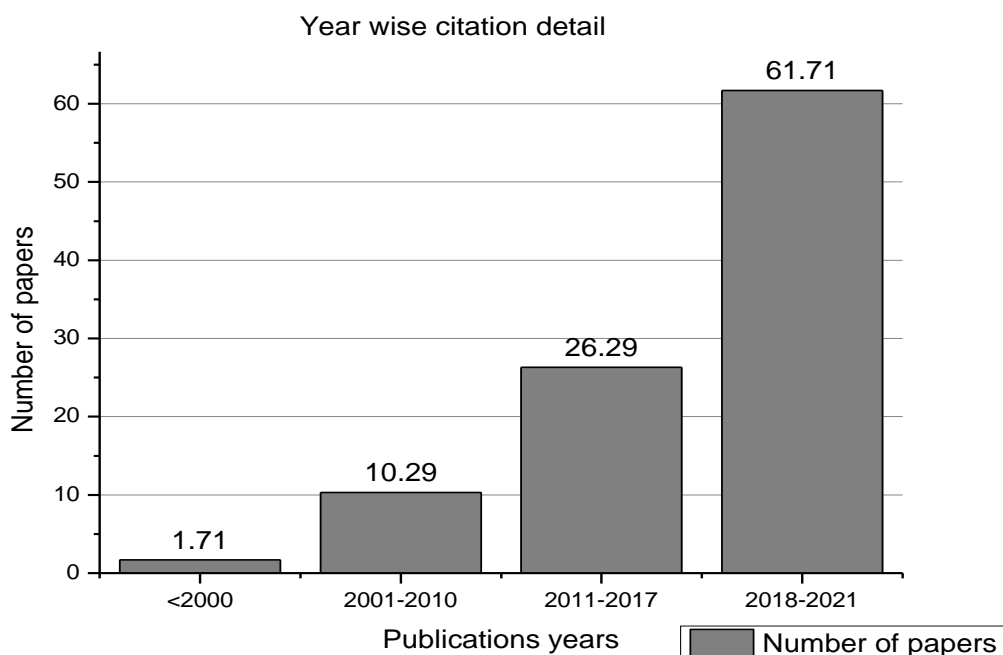


Figure 1. Year-wise citation detail.

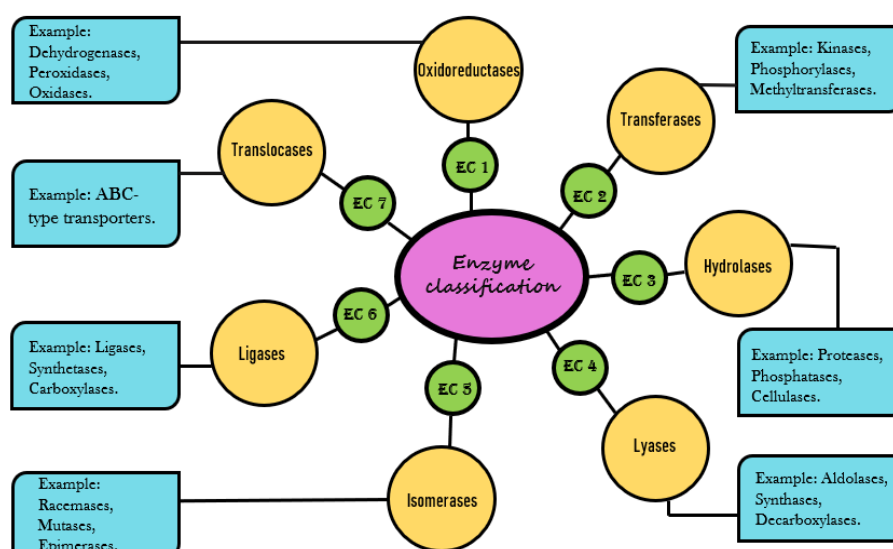


Figure 2. Enzyme Stratification.

3. Enzyme basics & Stratification

Enzymes have exhibited increased effectiveness and efficiency in many industrial processes and products, which has environmental benefits. This enzyme represents the protein groups and widely plays an important role in various processes like cell division, expression of genes, important immune system reactions, and metabolic activities. Enzymes are classified into seven classes, as shown in Figure 2 [29].

The enzyme oxidoreductase catalyzes the oxidation and reduction reaction by transferring the electron from one particle to another particle also removing the hydrogen atom utilizing the enzymes. These enzyme groups mainly utilize cofactors such as NADP⁺ and NAD⁺. This enzyme is found in algae, bacteria, fungi, plants, and animals. *Thermoascus aurantiacus*, *Neurospora crassa*, *Lentinus similis* like some fungi and bacteria such as *Enterococcus faecalis*, and *Lactobacillus kefir* are used to catalyze the lytic polysaccharide monooxygenases (LPMOs) substrates. It has applications in various sectors such as the immunological sector, medicine, and industries like fruit ripening and growth, cell wall metabolism, pathogens defense mechanism, etc., [30]. Examples are dehydrogenases, peroxidases, oxidases, reductases.

The enzyme class transferases catalyzed the transfer of a particular functional group (e.g., phosphate, acyl, and amino groups) from one substance to another. This enzyme is involved in the hundreds of various pathways in the biological reaction. It also involves the myriad cell reaction [29]. The Cytosolic Glutathione transferases (GSTs), which is a common transferase enzyme, are present in insects, plants, fungi, bacteria, and mammals. It is helpful in cancer monitoring and diagnostics, drug metabolism, drug, and pro-drug design, which is also used in the field biosensor for detecting the herbicide. The examples are trans aldolases, methyltransferases, kinase, transaminases.

Hydrolase enzymes catalyze the cleavage of the hydrolytic bond such as C-O, C-C, and C-N. This enzyme also transfers the water molecules; that is, the hydrolysis of the substrate can be catalyzed by the hydrolases enzymes. Few important examples of hydrolase enzymes are included below. This enzyme is mostly found in bacteria and filamentous fungi, like *Aspergillus niger*, *Trichoderma reesei*, and *Bacillus*, which are also present in some high-order organisms like gastropods, arthropods, plants, insects, and marine algae [29]. Hydrolase enzyme is used in all major industrial fields. Examples of hydrolase are protease, amylase, cellulase, and lipase.

Lyases enzymes catalyze the C-C, C-O, C-S, and C-N bond cleavage by eliminating the other bonds. Lyases can remove or add water, carbon dioxide, and ammonia elements from double bonds through the non-hydrolytic bond-breaking reaction. These enzymes are involved in the signal transduction pathways, anabolic and metabolic pathways, and DNA repair mechanisms [29]. It plays a key role in the food and chemical industries, preparation of natural products, and pharmaceutical intermediates. This enzyme presence was observed in plants, animals, and some microorganism's genera like *Fusarium*, *Aspergillus*, *Penicillium* [31]. Aldolases, decarboxylases, and synthases are examples of lyases enzymes.

The isomerase is the enzyme class that comes under the fifth group of enzyme commission (EC) classification (EC - 5). The isomerases catalyzed the isomerization reactions or intermolecular rearrangements. It is categorized into seven sub-classes: They were, racemases and epimerases, intramolecular oxidoreductases, intramolecular transferases, intramolecular lyases, cis-trans isomerases, isomerases altering macromolecular conformation.

The enzyme helps in interconversions which occurs in most organisms. For example, alanine racemase catalyzes the racemization of amino acids. Isomerases are subdivided into, cis-trans isomerases, intramolecular oxidoreductases, racemases & epimerases, intramolecular transferases, intramolecular lyases [32]. In most living organisms, the isomerase catalyzes the biochemical reaction, particularly carbohydrate metabolism, up to 4% [33]. They have important uses in biotechnology, drug discovery, and organic synthesis. For example, a novel glucose isomerase enzyme extracted from *Caldicoprobacter algeriensis*, has excellent thermostable property and gained industrial importance in recent years. And also, protein disulfide isomerase (PDI) had been overexpressed and helps in the proliferation of cancer cells. The anti-cancerous activity has been elicited on the Epithelial ovarian cancer models by targeting the PDI [34,35]. Examples are glucose isomerase, sucrose isomerase & D- arabinose isomerase.

The ligase is the class of enzyme that constitutes the sixth group of classification (EC - 6). It was further divided into six sub-classes based on the formation of bonds. They are phosphoric-ester, Carbon-oxygen, Carbon-nitrogen, Carbon-Sulphur, Carbon-carbon, and Nitrogen metal. The ligase catalyzes the joining of two or more molecules together, which are connected to the hydrolysis of analogous or ATP molecules. As they catalyze the reaction that generates the new molecule, they are termed Synthetases [36]. It involves biologically essential reactions, and it was about 81 ligases that play an important role in central metabolism [37]. For Example, DNA ligase is commonly used to join DNA fragments [38]. DNA ligase is an example of ligase.

An enzyme translocase can catalyze the molecules or ions translocation across the cell or separation in membranes which frequently hydrolysis the ATP, called translocases (EC - 7). The translocase is the new class of enzyme that catalyzes the translocation of ions/molecules within membranes or across the cell with the hydrolysis of ATP. It is also subdivided into six subclasses. By the translocated ion/molecule, they are divided into six subclasses. The enzyme catalyzing the translocation of hydrons, catalyzing the translocation of amino acids and peptides, catalyzing the translocation of inorganic cations, catalyzing the translocation of carbohydrates and their derivatives, catalyzing the translocation of other compounds, catalyzing the inorganic anions and their chelates belongs to this class [39]. It plays an essential role in the mitochondrial transport system. Its deficiency may lead to a disorder (i.e.) Carnitine-acylcarnitine translocase deficiency [40]. An example of a translocase enzyme is amino phospholipid translocase.

4. Enzymes in Industries

Amylase enzyme has very significant importance in the industrial sector. It is extensively used in detergent industries. The crude enzyme obtained from *Bacillus mojavensis* is used in laundry industries because of its alkaline conditions activity and stability at a wide temperature range & with other detergent components such as anionic & non-ionic surfactants and other oxidizing agents [41]. The Amylase soap-nut extract combination can de-stain the blood-stained cloth in 30 minutes [42]. Amylase is used in the detergent industry because of its sustainability and high stain-removing efficiency. At low temperatures, amylase, which is cold-active like amy175, is produced, and it has increased the power of stain removing efficiency [43]. Cold active amylases are used as an eco-friendly additive in detergents. Low temperature and higher pH are the suitable conditions for using amylase as a detergent additive

[44]. In the food industry, amylase will increase the quality and texture of the bread. The addition of microbial amylase with a specific volume (7.7%), reduces the hardness & chewiness by 11.5% and 17.2%, respectively. It also increased the texture profile by increasing the size and number of holes [45,46]. Amylase enzyme extracted from *Rhizomucor miehei* is used as a high potential candidate in food industries [45]. Multiple amylases from microbes and fungi were used in industries. Bacterial amylases are mostly used in many industrial processes due to their higher stability [47].

Amylase is used broadly in the production of bio-fuel. The second-wide application of amylase in the industry is bioethanol production. Initially, the slurry was gelatinized with a jet cooker. Then, it liquified using thermally stable α -amylase, which results in the saccharification and the release of fermentable sugars. It is subsequently fermented, and bioethanol is produced [48]. Using corn amylase instead of yeast in the process of dry-grind reduces enzyme usage, and it is combined and used in bioethanol production [49]. Bioethanol is produced from the biorefinery waste stream by treating enzymes like amylase with wheat bran using saccharification and fermentation methods [50].

Cellulase has an application in the pulp processing industry. Due to their potentials in paper pulp modification, the paper-making industries relied on lignocellulosic enzymes such as cellulase and xylanase. In combination with other enzymes, cellulase was used in bio-bleaching, deinking of waste papers, and in the modification of paper & pulp characteristics [51]. Cellulase is the third-highest enzyme used in industries for various applications. It is used in applications like bio pulping, bio stoning, bio bleaching, etc. In bio pulping, enzymes are made to break down, and by bio bleaching, the brightness of paper is increased [52]. Recycling cellulase with fresh cellulase helps to dissolve the pulp more effectively [53]. Lycopene & soluble dietary fibers (SDF) extraction using cellulase can reduce coronary heart disease risk, obesity, stroke, diabetes, and other diseases. Lycopene has antioxidant & anti-tumor properties. The enzymatic approach in the lycopene & SDF extraction from tomato will be considered safe when compared to chemical means. The use of cellulase and laccase will increase the yield of lycopene and SDF by 23.8% and 72.3%, respectively [54]. Cellulase was a cell wall degrading enzyme which has an application in the extraction of lycopene from tomatoes [55]. Extraction of soluble dietary fiber from potato pulp is first pre-treated with cellulase and xylanase, which gives better yield and also enhances the physiological and functional properties of SDF [56].

The enzyme protease is broadly used in leather industries. Leather industries follow the conventional process that uses hazardous chemicals such as sodium sulfide and lime. It results in issues such as effluent disposal and pollution. The protease is used to hydrolyze the non-collagenous protein of the skin and remove globulins & albumins [57]. Keratinolytic protease is an environmentally friendly biocatalyst that is used in the production of high-quality leather [58]. Proteases are used in place of traditional chemical agents, which reduces pollution [59]. Protease has important applications in the food and food processing industries. It is used in cheese ripening, flavor development, and milk coagulation in dairy industries and is also used in meat processing industries. In the cheese production process, proteases are added to milk in order to hydrolyze cheese. Proteases are also used in tenderizing meat [60]. In the baking industry, it is used in dough preparations and gluten development. In seafood processing, it is used in the production of fishmeal and enhances oil recovery [61]. Aspartic proteases are used in food and beverages [62].

Lipase plays an important role in degrading lipid pollutants. The presence of lipid pollutants in the water can cause severe problems like the formation of the lipid layer over the water, which affects the aquatic ecosystem. Similarly, the lipid pollutants present in the soil can cause difficulties in water movement, reduce the ability of water to bind to soil particles, and limit the aeration, which adversely affects the ecosystem. The lipase, which is immobilized with cellulosic particles, was used to degrade the lipids and grease accumulated in wastewater pipelines [63]. To degrade lipid accumulated oil bodies, TAG (triacylglycerol) lipases are responsible for the lipid productivity enhancement in microalgae [64].

Due to the esterase solid remediation property, it can degrade the herbicides such as metsulfuron-methyl, chlorimuron-ethyl, and tribenuron-methyl. The immobilized esterase can be used in the remediation of soil contaminated with pesticides [65]. The calorimetric method is used to determine the soil's esterase activities in which fatty acids esters are linked to p-nitrophenol as substrates [66]. Novel esterase, which was derived from metagenomics, plays a major role in diesel-oil degradation [67]. Enzymes are catalytic biosensors that bind to the analyte & converts into products. By the electrochemical transducer, read out the target consumption during the conversion. The enzyme will be selected based on the target, such as acetylcholine esterase for acetylcholine, cholesterol oxidase for cholesterol, glucose oxidase for glucose, horseradish peroxidase for H_2O_2 , and tyrosinase for bisphenol. Most of the enzymes act as a biocatalyst as well as a bioreactor for many biological processes, such as H_2O_2 acts as a signaling molecule for cell death monitoring, immune cell activation, root growth, and stomatal closure. So, it is important to biosensing in many platforms [68].

5. Clinical usage of enzyme

Currently, enzymes have profound application in the clinical section and an important role in diagnosis, prevention, therapeutics, and biochemical analysis. Nowadays, in the therapeutic field, enzymes are used to treat digestive disorders to cancer therapy, also cardiovascular and lysosomal storage diseases. The recent advanced techniques improve the production of human-like therapeutic enzymes by DNA recombination [69].

Amylase has the capability of targeted drug delivery and is also used for "Smart release". Amylase taken from *Aspergillus oryzae* is used as a digestive aid to treat dyspepsia [70]. For pancreatic disease diagnostics, primarily amylase is used [71]. The exponential increase of multidrug-resistant bacteria happened throughout the year and became a severe threat to human health. These microorganisms form a biofilm which is extracellular polymeric substances (EPS) that helps to attach to the biotic and abiotic surfaces. On successful conjugation of silver nanoparticles (AgNPs) with α -amylase, it is used against *K. pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA). It exhibited antimicrobial activity and reduced the formation of biofilm [72]. The higher levels of α -amylase in the liver could be used to indicate the earlier stages of obesity [73]. The α -amylase in the serum can be used as a biomarker for various pancreatic ailments. And α -amylase concentration can be used as real-time detection for pancreatic disorders. Millions of people can get benefitted from this affordable procedure [74]. Despite the benefits of kidney transplantation, complications in the post-transplant period affect the long-term allograft. For most kidney transplants, "Delayed graft function (DGF)" is a common complication. It is caused particularly for those who received a kidney transplant from a deceased donor [75]. At

the earlier stages of DGF, an increase in serum amylase and Resistive index (RI) will occur. DGF can be detected earlier by the serum amylase and $RI > 0.7$ after the kidney transplant [76].

Cellulase has a vital clinical application. The bezoars are caused by the presence of partially digested or undigested material in the gastrointestinal tract. It is cured by cellulase, which prevents reintervention also [77,78]. The cellulase-treated wheat bran used in pasta production has comparatively high soluble fibers and a lower glycaemic index than untreated [79]. Keratitis is a serious corneal infection that may cause blindness. The use of contact lenses is the leading risk factor for microbial keratitis. In combination with anti-amoebic compounds, cellulase effectively prevents keratitis, which targets the cyst wall [80,81]. In medical textiles, antibacterial activity was necessary to reduce the bioburden and hospital-acquired infections. The cellulase-treated fabrics were coated with Zinc Oxide (ZnO) nanoparticles, which provide better adhesion, and the antibacterial activity remains even after the multiple washing [82]. The extracts from cellulase-treated microalgae cells show higher toxicity against the benign tumor than lysozyme and other chemical drugs [83].

Protease (Bromelain, chymotrypsin) from *Ananas comosus* and serum was used to treat edema, inflammation, upper respiratory tract, and ophthalmology diseases. Papain protease is used for the treatment of Thrombotic disorder [84]. Proteolytic activity regulation is a major factor in the clinical application of protease. Cellular receptors, growth factors, and chemokines are regulated by protease through inactivation and activation of gene regulation and intracellular signaling [85]. Protease was also investigated for the nanomedicine approaches to target and treat the tumor, and some fundamental processes were already implemented to treat cancer disease [86]. The maintenance of homeostasis is essential in pathogenic organisms and humans. This can be done by protease enzyme [87].

Lipase enzyme has an excellent pharmaceutical application and diagnostic aids. Especially, it is used for the digestion of fats in humans also for the treatment of lifestyle diseases known as obesity [88,89]. The lipase from *Candida rugosa* synthesizes lovastatin, a drug that lowers serum cholesterol levels. 4-Methoxyphenyl glycidic acid methyl ester (MPGM) is a key product synthesis from diltiazem hydrochloride is utilized for the coronary vasodilator screened from *Serratia marcescens* [90]. Enantioselective transesterification and interesterification response by the assistance of lipases have incredible essentialness in the drug industry for specific diacylation and acylation response [91]. Monoacylglycerol lipase is responsible for the pro-tumorigenic or pro-inflammatory effects of the metabolism process [92].

Esterase plays a major role in clinical applications. Cocaine esterase (CocE) is a favorable strategy for treating addiction and overdose cocaine [93]. Leucocyte esterase enzyme is widely found in urine and feces. The strip made by leucocyte esterase plays a major role in periprosthetic joint infection diagnostics [94]. It plays a main role in inflammation reduction, pain relief from arthritis, menstruation, fever, and sunburn. Esterase extracted from *Trichosporon brassicae* has effective pain-relieving results. *Pseudomonas* sp. producing esterase enzyme has an ibuprofen-like therapeutic effect [95]. Feruloyl esterase will increase the antioxidant property when it is added to other compounds like fruit juice, and syrup also plays a role in flavor and color [96].

Table 1. Different sources of enzymes with allied industrially significant details.

Enzyme name	Organism name	Growth media	Specific conditions	Source	Enzyme activity	Reference
AMYLASE	<i>Streptomyces parvulus</i> strain sankarensis-A10	Starch agar medium which has the composition of g/L: soluble starch 10.0, seawater (50% v/v), Meat extract 3.0, agar 15. Then, the pH adjusted to 7.0 ± 0.2	Incubated at 28 ± 2 °C for 7 days.	Vishakhapatnam coast, Bay of Bengal (Sediment sample)	25.53 ± 0.50 U/ml.	[97]
	<i>Bacillus subtilis</i> SUNGB2	Thermus agar added with agar, Beef extract, peptone, yeast extract, and NaCl.	Incubated at 45 °C for 24-48 hours.	Dusun Tua Hot Spring, Hulu Langat, Selangor & Sungai Klah Hot Spring, Perak, Malaysia (Water samples)	22.14 U/ml.	[98]
	<i>Streptomyces</i> sp. Al-Dhabi-46	Actinomycetes isolation agar	Incubated at 28 °C for 7 days.	Jazan, Saudi Arabia (Soil samples)	241 ± 18.1 U/ml.	[99]
	<i>Bacillus</i> sp. strain SP-CH7	Horikoshi medium	Incubated at 37 °C for 72 hours.	Chilika Lake, India (Sediment samples)	202.857 U/ml.	[100]
	<i>Bacillus</i> sp. Q-164	Nutrient agar medium	Incubated at 37 °C for 48 hours.	Vishakhapatnam, Andhra Pradesh, India (Palm wine)	942 U/mg.	[14]
	<i>Saccharopolyspora</i> sp. strain A9	Glycerol yeast extract agar, Maltose yeast extract agar, Glucose asparagine agar, Starch casein agar medium containing Starch (Prepared in artificial seawater)	-	Goa, Alibagh, and Mumbai coastal region of India (sediment samples)	1640.80 U/mg.	[101]
	<i>Bacillus tequilensis</i> TB5	Starch agar medium	Incubated at 37 °C for 72 hours.	Chandigarh, India (Vegetable waste)	39.736 ± 0.296 U/ml.	[102]
CELLULASE	<i>Bacillus subtilis</i> D19	Soluble starch	-	Food sample	0.670 U/mg.	[103]
	<i>Bacillus pseudomycolides</i> Y3	Carboxyl methylcellulose (CMC) Agar medium	The plates were incubated at 37 °C until sufficient growth.	Rajshahi Sugar Mills Ltd., Bangladesh (Sugarcane bagasse)	7.82 IU/ml.	[104]
	<i>Bacillus velezensis</i>	Primary screening medium (carboxymethylcellulose sodium, NaCl, Tryptone, yeast powder)	The plates were incubated at 37 °C for 24 hours.	Lanxi Pig Farm, Suihua City, Heilongjiang Province, China (Manure samples)	20.20 ± 0.74 U/ml.	[105]

	<i>Saccharomyces cerevisiae</i> SCPW 17	Media constituents were NaNO ₃ , KH ₂ PO ₄ , MgSO ₄ .7H ₂ O, KCl, Protease, peptone, Agar, aqueous glucose, carboxymethyl cellulose, and xylan. & Ligninase basal medium supplemented with lignin, agar.	The pH of the medium was 6.0 and plates were incubated at 28 ± 2 °C for 3-5 days.	Mushroom farm in Yala Local Government Area of Cross River State, Nigeria (Soil samples)	0.01951 ± 0.32 U/mg	[106]
	<i>Bacillus Sp.</i> DNH5437	Tryptic soy agar & Modified M-II medium (K ₂ HPO ₄ , KH ₂ PO ₄ , KCl, NaCl, NH ₄ Cl, MgSO ₄ .7H ₂ O, CaCl ₂ .2H ₂ O, peptone, yeast extract, glucose, agar)	At 30 °C the plates were incubated and 37 °C for a month.	Gut sample of <i>O. coeruleus</i> collected from North East Iran (Almond gardens).	9.0 U/mg.	[107]
	<i>Bacillus licheniformis</i> NCIM 5556	Basic Liquid Media (KH ₂ PO ₄ , (NH ₄) ₂ SO ₄ , MgSO ₄ .7H ₂ O, FeSO ₄ , NaCl & yeast extract.)	-	Rajapur (Western Coastal area) Ratnagiri District, Maharashtra, India. (Soil and water samples)	42.99 U/ml.	[108]
	<i>Aspergillus fumigatus</i> (CWSF-7)	Potato dextrose agar medium	-	Bhubaneswar, India (Soil samples)	1.9 U/ml.	[109]
	<i>Bacillus velezensis</i> ASN1	Carboxymethyl-cellulose (CMC) agar medium	For 48 hours at 37 °C the plates were incubated.	Barka, Oman, Animal farmhouse. (Soil samples)	2.42 U/ml.	[110]
	<i>Bacillus subtilis</i> BY-2	LB agar medium supplemented with 1 % CMC	The incubated time is about 37 °C for 24 hours.	Tibetan pig from Shaanxi HuaYi Industrial Co., Ltd.; Tibetan pig breeding base. (Intestinal samples)	3.56 U/ml.	[111]
	<i>Cladosporium cladosporioides</i> NS2	Potato dextrose agar medium.	For 4 to 6 days the plates were incubated at 45 °C.	Agriculture field of Banaras Hindu University, Varanasi, India. (Rotten wood sample)	0.240 U/mg.	[112]
	<i>Aneurinibacillus aneurinilyticus</i> BKT-9	Nutrient agar medium	The medium was incubated at 37 °C.	Dal lake, Jammu & Kashmir, India. (Water samples)	83.1092 U/L.	[113]
PROTEASE	<i>Vibrio alginolyticus</i>	Skim milk media	The culture plate maintained and kept for incubation for 21, 24, 27 hrs at 30°C.	Sediments sample collected from Pantai Gading mangroves, North Sumatra, Indonesia.	228.81 U/ml.	[114]
	<i>Citricoccus sp.</i> (KC522120.1)	Alkaline agar media	Specific temperature 24-48hrs was maintained for the culture plate at 30°C.	Agriculture soil from Regional Centre of Soil Salinity Research Institute, Lucknow (U.P.), India.	26.87 U/ml.	[115]

	<i>Streptomyces</i> sp. Al-Dhabi-49	Skimmed milk agar media	The plates kept at 28°C for 5 days up to pH-9.	Soil sample collected from Dammam marine region.	147.2 ± 3.6 U/ml.	[116]
	<i>Bacillus cereus</i> TSA5	Nutrient Agar media	The growth of this bacteria is high at 32°C for 2 days at pH 7-8.	Agriculture, compost, and garden soil	60.41 ± 0.01 U/mg.	[117]
	<i>Streptomyces</i> sp. Al-Dhabi-82	Starch casein agar	To reduce the microbial growth nalidixic acid and nystatin-like antibiotics were added with the starch casein agar.	Jazan region of Saudi Arabia soil.	276 U/mg.	[118]
	<i>Pseudomonas lundensis</i> DZ845	Azocasein with phosphate buffer saline solution and sodium azide	The specific temperature maintained for the samples were 37°C for 24 hours.	Raw milk samples from New Zealand.	1.04±0.02 U/ml.	[119]
	<i>Pseudomonas aeruginosa</i>	Modified Luria-Bertani (MLB) agar plates	Additionally, 10% of toluene and cyclohexane were added to the medium.	Crude oil contaminated soil from Jiangsu province, China.	10,876 U/ml.	[120]
	<i>Nesterkonia halobia</i>	casein-yeast plate agar (CYP)	48 hrs incubation at 37°C recommended.	Soil or mud from the Lake Abjata shore, a soda alkaline lake, Ethiopian Rift valley.	41.2 U/mg	[121]
	<i>Chromobacterium violaceum</i>	Using slaughterhouse effluent which filtered (500ml) and granulated agar (7.5 g) slaughterhouse effluent agar was prepared.	-	From Ramanthpur of Telangana, the slaughter house effluent was collected	0.1254 U/ml.	[122]
	<i>Haloferax lucentensi</i>	Tryptone Yeast Extract (NTYE) agar.	25% of NaCl was used	Agro-food waste.	142.34 U/ml.	[123]
	<i>Bacillus</i> sp. SP II-4	Skim milk agar.	-	Saltpan where the strains were collected which is located in Kanyakumari	591.04 U/mg.	[124]
LIPASE	<i>Pseudomonas aeruginosa</i>	Nutrient agar	-	Soil from mechanic's shop inverse Forestry College, Jericho, G.R.A, and Ibadan.	528,540 U/ml	[125]
	<i>Pseudomonas aeruginosa</i>	Nutrient broth	20% (v/v) to 40% (v/v) wastewater added to the broth.	The wastewater produced from oil processing plant located in Tehran.	0.76 U/ml.	[126]
	<i>Pseudomonas helmanticensis</i> HS6	Yeast Extract Peptone Dextrose Agar (YPDA)	This media was incubated for 48 hrs at 30°C to 37°C.	From Sikkim, the soil sample were collected above sea level ranging 2500 to 4272 m.	179.3 U/mg.	[127]

	<i>Pseudomonas reinekei</i>	Inoculated in a medium containing olive oil is a carbon source.	Incubated at 28°C for 72 hrs.	Wicklow mountains soil from Ireland.	3.18I U/mg.	[128]
	<i>Pria Laot Sabang 80</i> (PLS 80)	The solid medium of ½ Thermus (½ T)	Kept for 70°C for 18 hrs.	The water sample was collected from underwater fumaroles in Aceh Province, Indonesia.	54.2 U/mg.	[129]
	<i>Cystobasidium oligophagum</i> JRC1	YPD medium	Incubated at 30°C for 120 rpm.	Soil collected from Jodhpur is rich in cellulosic waste.	2.88 ± 0.166 U/mg.	[130]
	<i>Staphylococcus warneri</i>	Luria Broth (LB) agar	-	Samples from sludge and sediment were taken from the Pulp and Paper Mill of Uttarakhand.	2.3 U/mg.	[131]
	<i>Psychrobacter immobilis</i>	Nutrient marine medium 2216	Kept for incubation at 4°C.	Seawater collected from Frei Montalva Base at King George Island.	1.78 U/mg.	[132]
	<i>Staphylococcus saprophyticus</i>	Nutrient agar with seawater.	Plates were incubated at 37°C for 5 days.	Sediment was collected from the eastern slope of the Arabian Sea.	100 ± 0.121 U/mg.	[133]
	<i>Aspergillus awamori</i>	Malt extract (ME) fungal agar medium.	2% (v/v) of rice bran oil was added with the medium.	The fungal strain from the Arabian sea of Kerala was isolated.	123.7 U/mg.	[134]
	<i>Fusarium solani</i> FS1	Potato-dextrose-agar	Plates were maintained 4°C.	Federal Rural University, Plant health department, Pernambuco.	0.45 U/mg.	[135]
ESTERASE	<i>Micropolyspora faeni</i>	V8 agar	Kept incubation at 40°C for 6 days.	From London School of Hygiene and Tropical Medicine the soil sample were collected.	145 U/mg	[136]
	<i>Bacillus</i> sp. 4	Castenholz basal salts solution	The plates were kept for 24 h at 65 °C on a shaker at 150 rpm.	Alangüllü thermal spring (Aydin, Turkey)	137.77 U/ml.	[137]
	<i>Rhodococcus</i> sp. LKE-021	Nutrient broth	Using 135 rpm at 60°C.	Soil sample from Gangotri, Uttarakhand, Himalayas.	795.1 U/mg.	[138]
	<i>Rhodococcus</i> sp. LKE-021	Nutrient broth	Maintaining in 60°C for 5 days.	Samples of soil from Gangotri, Uttarakhand Himalayas.	13.5 U/mg.	[25]
	<i>Ophiostoma piceae</i>	Malt extract –glucose –agar	This culture kept incubated at 26°C and 160 rpm.	-	79 U/mg.	[139]
	<i>Pseudomonas putida</i>	Luria-Bertani	Kept at 30 °C for the period of 1 to 2 days.	Soil from National Taiwan University and the Taoyuan District was collected.	1.00±40 U/ml.	[140]
	<i>Trichoderma viride</i>	Nutrient agar	-	Cultivated area clay in western Washington was collected.	0.27 U/mg.	[141]

6. Diverse sources of enzyme

Microorganisms are extensively used in industries as they afford enormous economic ease to industries. Enzymes produced by animals and plants are the most favored source because of their advantages like easy and consistent production. Microbial enzymes are more stable than plant and animal enzymes. Enzymes extracted from microbes are easier to handle and cheaper than plant and animal sources. Microbial enzymes can be produced very effectually by different fermentation techniques in a frugal manner with less time and space demand.

Production of microbial enzymes on a large scale can be done facilely. The samples are being selected from different places to know the qualitative or quantitative nature of the microbes. The growth media of enzymes, conditions, source, and activities are discussed below (Table 1).

7. Recent process advancements in enzyme technology

Due to its wide range of applications, amylase production needs to be increased. The important step in the production of enzymes is fermentation technology. For amylase production, submerged fermentation and flask scale fermentation are suggested because of their easy handling and control of greater ecology factors such as pH and temperature [142,143]. In the submerged fermentation, the maximum activity is analyzed under the optimum condition. Mainly solid-state fermentation is utilized for the production of the metabolites where the purity requirement is low. But also, the down streaming process involved in the SSF is more challenging [144]. To obtain the pure polishing amylase enzyme product, a series of downstream processing is involved. They are ultrafiltration, precipitation, membrane separations, and chromatography techniques [145].

Owing to its industrial application, cellulase manufacture is essential to increase. After the isolation process was carried out, the submerged and solid-state fermentation techniques were utilized to produce cellulase. A study shows that the submerged fermentation was used to increase cellulase enzyme production [146]. Among the various factors involved in the submerged fermentation, the incubation time and temperature play a wide role in cellulase production. But the changes made in submerged fermentation steps and factors can directly affect the production of the enzyme [147,148]. And the purification steps involved here are ammonium sulfate precipitation, centrifugation, dialysis, and chromatography techniques like anion exchange chromatography, gel filtration chromatography [149,150]. After the downstream process, the purity of the product and activity can be analyzed.

Nearly 60% of the industrial market needs protease enzymes. In order to satisfy, the production needs to be raised. To raise the production needs, sources from various organisms were selected, incubated, and characterized; the protease production using the Submerged fermentation (SmF) or Solid-state fermentation (SSF) step can be carried substrate is liquid, the shake flask fermentation can carry. The shake flask fermentation used for the production of protease in a liquid medium gives a high amount of yield, but it undergoes several purification steps that are difficult to carry out [151,152]. And it also consumes much time. The production of protease can be influenced by pH, temperature, incubation period, and nitrogen concentration. In these above methods, the widely explored process is wheat bran

mediated SSF because of its easy purification steps and high yield production of protease [153]. Down streaming represents obtaining pure products and product polishing. A wide range of techniques is available to recover the product from the fermented substrate. Protease purification involves several steps and procedures like precipitation, liquid-liquid extraction, chromatography, etc. [154]. To purify protease Diethylaminoethyl cellulose (DEAE-C) column chromatography, gel filtration technique, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is used. Among these techniques, DEAE-C column chromatography is used tremendously in the past few years to reduce the number of steps involved in purification [155,156].

Lipase versatility makes this a preferable choice for potential application in the pharmaceutical and cosmetics industries. After the strain selection, improvement, and media optimization, fermentation technology was carried out. SmF is mostly used to produce lipase, SSF can also be used rarely. Both SMF and SSF are utilized for the production of largescale bioactive compounds [157]. The nitrogen and carbon concentration, temperature, pH, and dissolved oxygen concentration may influence lipase production [158].

In some cases, immobilized cell culture is also used, but the submerged fermentation is a technique that is employed for the controlled microorganism cultivation in the liquid substrate and target for high yield formation. Recent studies show that the preferred choice for lipase fermentation is SSF because it is cost-effective, simple, easy purification, and eco-friendly [159]. Purification is used to evaluate the enzyme's stability, activity, and commercial value. There are so many techniques for purifying the enzyme-like chromatography, precipitation, flocculation [160]. In this technique, pre-purification steps like ultra-purification and so forth are needed. To overcome the above issues, numerous research works are carried out continuously by industrial researchers. Recent technologies applied for lipase purification are immuno-purification, column chromatography, and hydrophobic interaction chromatography. Hydrophobic interaction chromatography is considered preferable among these techniques because the lipase enzyme is hydrophobic, so lipase purification is achieved by hydrophobic interaction chromatography [161].

Esterase can degrade industrial pollutants, so the enzyme needs to be produced immensely. Fermentation was carried after the preparation of the sample. There are three techniques involved in esterase fermentation. They are submerged, solid-state, and slurry state [162]. Among the above-mentioned three methods, SmF supplement with olive oil and maltose gives high production of esterase enzyme [163]. These crude enzymes are purified using several techniques to obtain the maximum amount of purified esterase enzyme [164]. This purification step includes various processes like ammonium sulfate precipitation, dialysis, acid hydrolysis, and chromatography like gel filtration chromatography, column chromatography [165].

8. High throughput enzyme assay

Metagenomics is a technique that studies most of the microbes and the enzyme related to the microbes isolated from an environment. It is a powerful technique for novel gene discovery and provides an opportunity for innovative biotechnological process development. Recently, it was used in the discovery of novel biocatalysts. Up to 99% of the uncultured bacteria were explored using this technique. Industries depend highly on enzymes for the catalytic process because of the increased reaction rate to several folds. In addition to this, it

helps in finding superior catalysts for numerous industrial applications [166]. Metagenomics is involved with two ways of approaches for screening the biomolecules. They are sequence-based and functional-based screening of metagenomic libraries. It surpasses the technique that needed the bacteria to be isolated and cultivated. The genomic DNA was isolated directly from samples. It was found to be effective in tapping the metabolic and genetic diversity of complex ecosystems. It helps in the identification of bioactive molecules and novel enzymes [167].

The new approach for efficient amylase activity detection was studied. Glucose oxidase was commonly used in glucose monitoring systems because it is highly specific to glucose. It works based on the principle that the glucose oxidase and the products of amylase interact in the presence of O_2 , which results in the formation of gluconic acid and H_2O_2 . Later, the produced H_2O_2 can be detected using a kit (Phenol, 4-aminoantipyrine, and peroxidase) and results in red color formation. Further, the absorbance will be measured. Due to the reliability & applicability of this method, the α -amylase activity can successfully determine [168].

The transcriptional regulator-based biosensors can specifically interact with their effectors and produce measurable signals by using the specific products from enzymatic reactions. The Genetic enzyme screening system, massive libraries were utilized by a biosensor to find novel enzymes. The Cellobiose detectible genetic enzyme screening system, a whole-cell biosensor, can detect cellulolytic activity and reported that it was a powerful tool because of the high sensitivity in the presence of Cellulosic substrates. The biosensor consists of the regulator and reporter fluorescent protein for detecting the cellulase activity in live cells [169].

The energy transfer-based biosensors were most commonly used to detect proteolytic activity detection in real-time, and they are highly sensitive & wash-free. As a result of long-range dipole-dipole interactions, energy transfer will occur. So, it can be used in in-vivo conditions. They are used in the early diagnosis of severe diseases [170].

Various studies on lipase enzyme activity prediction were made in recent years. Among those, the lipase activity determined by the aggregation-induced emission-based fluorescence turn-on assay is very notable. The terphenyleneethyne (TPE) derivative with $-COOC_6H_{13}$ can be hydrolyzed into TPE-COOH, which aggregates easily and has low solubility. A novel fluorescence turns on the probe, which has the ability to detect aggregation-induced emission to visualize the lipase activities. It has high sensitivity and has the application for real-time detection of lipase activity [171].

The enzyme works more in the activities of all organisms, from cell division & growth to aging & death. Abnormal activity of enzymes leads to dysfunction and disease. So, it has great significance in the diagnosis and also in the disease treatment. An ultrasensitive technique for detecting T4 polynucleotide kinase (T4 PNK) and telomerase has significant importance. It works based on the primers continuously extending to produce more activation regions, resulting in the initiation of DNase activity of the CRISPR/Cas12a. Then, fluorescent assay ultra-sensitively or visually detects the T4 PNK and telomerase activity. When compared to other methods of detection of these enzymes, it was ultrasensitive, fast, and visual detection of enzyme activity is feasible [172].

The enzyme activity analysis is needed for understanding the pathways associated with disease at the molecular biology level. The construction and implementation of nano kits were helpful in the cost-effective analysis of enzyme activity within the living cells. A nanometer-sized capillary tube containing the working electrode and the kit components has to be inserted

into it for protein analysis. For enzyme activity analysis, reversed electrochemical pumping can be used to confine the targeted organelle in the nanocapillary tip [173].

Recent advancements in chemical proteomic methods facilitate enzyme activity detection and quantification. The activity-based proximity ligation (ADPL) method was used to detect and quantify the enzyme activity in single cells. The ADPL platform uses direct conjugation formats, which enables amplification and quantification of active enzymes, even in subcellular resolution [174].

Matrix metalloproteinases-9 (MMP 9) play a vital role in tumor metastasis & cancer cell invasion. It is commonly used as a potential biomarker for different cancers such as breast cancer, cervical cancer, bone tumor, hepatocellular carcinoma, pancreatic cancer, lung cancer, ovarian cancer, and Osteosarcoma. For the detection of MMP 9, researchers developed an electrode-free electrochemical biosensor. Methylene Blue (MB) was conjugated to the N-terminal of this specific substrate peptide and was immobilized on the Au electrodes. In the presence of MMP 9, it will cleave this peptide. This cleavage results in the release of electroactive MB peptide and changes the electrical tunneling current. It can be used in the detection of MMP 9 [175].

9. Conclusions

There is a perpetual hunt for enzymes as it is widely used, particularly in industries. Enzymes are widely used in key fields, such as pharmaceuticals, food processing, and even as substitutes for chemical additives. This review strives to overview amylase, cellulase, protease, lipase, and esterase and their industrial and clinical application. For this review, fonts are collected from various supreme journals and publications. Sources used and the method of production is also described in this review. The reason for using enzymes is that it is a biocatalyst without any side effects and reduces time consumption. Industrial consumers use it every day to create marketable products. Industries are in need of enzymes because of their low processing time. But the industries are facing difficulties in enzyme production and its purification. The recent advanced methods for enzyme production and purification discussed in this review may help researchers and industrialists choose the best, most effective, and suitable techniques for their specific approaches. Nowadays, there is a necessity for new and more versatile enzymes. For the research purpose, this review will give full thought about enzyme production from the microorganism. This allows us to understand the future perspective of enzymes and their importance in the industrials sectors. The further year should see a lot of trends in enzyme and its application.

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Conflicts of Interest

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