

Review

Lignocellulosic Biomass and Enzymes: Fundamentals, Emerging Technologies, and Applications

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Abstract

Lignocelluloses are complex plant polysaccharides made of lignin, cellulose, and hemicellulose, and products made from these components find immense market potential. They can be effectively valorized to products related to bioenergy, bioplastics, food and nutrition, medication delivery systems, and other elements. Significant sources of lignocellulosic biomass include sugarcane bagasse, corn cob, rice straw, potato haulms, cocoa pods, etc. Enzyme-based valorization processes find immense potential, as they are eco-friendly and sustainable. A few prominent enzymes being used in the process, include hemicellulases (3.2.1.X), ligninases (EC 1.11.1.14), cellulases (EC 3.2.1.X), etc. These enzymes can be obtained from a diverse group of microorganisms and may be utilized in various industrial processes. The present review accounts for prominent lignocellulolytic enzymes, microbes producing these enzymes and their specific industrial applications. The review also highlights advances in enzyme production strategies and their production processes.

Keywords

Ligninolytic enzymes; xylanases; hemicellulases; cellulases; microbial enzymes



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1. Introduction

Growing demand for renewable energy and sustainable industrial processes has driven the need for efficient strategies to reuse and recycle numerous resources and one among them is recovering lignocellulolytic enzymes. Lignocellulolytic enzymes are a diverse group of biocatalysts that play a crucial role in the degradation of lignocellulosic biomass, one of the most abundant renewable resources on Earth [1]. This complex biomass consists primarily of three major components: cellulose (40-50%), hemicellulose (25-30%), and lignin (15-20%), which together form the intricate structure of the plant cell wall [2]. The recalcitrant nature of lignocellulose poses significant challenges for its efficient utilization in various industrial processes [3]. Lignocellulolytic enzymes have evolved to overcome these challenges by synergistically breaking down the complex polymers into simpler, more manageable compounds [4], facilitating their use in various biochemical and microbial processes. This enzymatic breakdown is fundamental in transforming plant biomass into valuable products such as biofuels, chemicals, and other renewable materials. The three main categories of lignocellulolytic enzymes are cellulases (EC 3.2.1.X), hemicellulases (EC 3.2.1.X), and ligninases (EC 1.11.1.14), each targeting specific components of lignocellulose [5, 6].

Cellulases hydrolyze cellulose, the most prevalent polysaccharide, converting it into glucose for subsequent fermentation into biofuels [2]. This group includes exoglucanases (EC 3.2.1.91), which attack cellulose chain ends, endoglucanases (EC 3.2.1.4), which cleave internal bonds, and β -glucosidases (EC 3.2.1.21), which further break down smaller sugar molecules. Hemicellulases (3.2.1.X) target hemicellulose, a complex, branched carbohydrate of sugars like xylose and mannose. Examples include xylanases (EC 3.2.1.8) and mannanases (EC 3.2.1.78), which degrade xylan and mannan, respectively, into simpler sugars. Ligninases (EC 1.11.1.14), such as laccases and peroxidases, work on lignin, a robust aromatic polymer, breaking it into more manageable compounds [3].

These enzymes are produced by diverse microorganisms, particularly fungi and bacteria [7]. Prominent fungal producers include species like *Trichoderma reesie* and *Aspergillus niger*, while bacteria such as *Clostridium thermocellum* and *Bacillus subtilis* also generate cellulases and xylanases (EC 3.2.1.8) [8]. Certain actinomycetes, like *Streptomyces*, are known for producing a high yield of lignin-degrading enzymes. In recent years, extremophilic microorganisms have gained attention as sources of lignocellulolytic enzymes with unique properties and expanding the potential applications of these biocatalysts in various industrial processes [9, 10]. The wide-ranging applications of lignocellulolytic enzymes make them essential in multiple industries. In biofuel production, they aid in converting plant biomass into fermentable sugars [2]. They also play a significant role in bioremediation by breaking down organic waste. In the paper and pulp industry, these enzymes help reduce the need for harsh chemicals during bleaching [11]. Additionally, they are incorporated into animal feed to enhance the digestibility of fibrous plant materials. Overall, lignocellulolytic enzymes are indispensable for the efficient utilization of plant biomass and the advancement of sustainable bioprocesses, contributing significantly to the development of eco-friendly industrial practices and renewable resource utilization [5].

Although lignocellulosic enzymes can break down plant matter, they have many limitations that include low efficiency, high cost, and less stability. Their production, stability and reaction to inhibitory compounds within the biomass are significant hurdles. The lignocellulolytic enzymes,

which are currently in use, are inefficient in catering for the complete breakdown of complex lignocellulosic structures, leaving significant biomass unutilized.

One of the most important challenges is that the production of these enzymes is expensive, thus limiting their widespread industrial applications. They are sensitive to temperature, pH fluctuations, and product inhibition, requiring exact control to maintain optimal activity. There is a requirement to involve technological advancements, such as in engineering enzymes and a better understanding of enzyme complexity and enzyme mechanisms. Modifying the enzymes' properties to improve stability, substrate specificity, and tolerance to inhibitors is required. There is an excellent need for environmentally friendly pretreatment methods and an optimized process for enhanced production through alternative pretreatment and production strategies.

Optimization of cost-effective and highly efficient production mechanisms is a significant challenge in the case of production, wherein lignin barrier, pretreatment cost, byproduct management, lignin degradation, and managing accessory proteins need to be addressed.

The present review gives a detailed description of the existing scenario of lignocellulolytic organisms, their mode of action, and technological advances in production and application processes. Lignocellulolytic enzymes find numerous industrial applications and thus find great market potential. The industrial enzymes market, including lignocellulosic enzymes, is projected to grow at a Compound Annual Growth Rate (CAGR) of 7.2% from 2024 to 2029. This review highlights the industrial applications of lignocellulolytic enzymes and technological advances for enhancing their production.

2. Lignocellulolytic Enzymes

Lignocelluloses are precisely composed of lignin, cellulose, and hemicellulose, and each of these fractions can be modified and valorized to numerous value-added products. One of the most promising tool working in the valorization process are enzymes. Lignocellulolytic enzymes are a cluster of biocatalysts actively participating in the process, and some of them include cellulases (EC 3.2.1.X), xylanases (EC 3.2.1.8), pectinases (EC 3.2.1.15), arabinases (EC 3.2.1.99), etc. This section of the manuscript covers a description of lignocellulolytic enzymes.

Cellulases (EC 3.2.1.X) hydrolyze cellulose to glucose and are classified into three types (Endoglucanases, Exoglucanases, β -glucosidases). Endoglucanases catalyze random cleavage of internal β -1,4-glycosidic bonds within cellulose chains and work in reducing polymer length and targeting amorphous regions. Exoglucanases, or cellobiohydrolases, progressively remove cellobiose units from the ends of cellulose chains and are primarily associated with activity against crystalline areas more resistant to enzymatic attack [12-15]. β -glucosidases catalyze cellobiose and short-chain oligosaccharides to glucose, which is necessary to avoid feedback inhibition from accumulated cellobiose and for effective breakdown of cellulose to fermentable sugars.

They are produced by various types of bacteria, actinomycetes, and fungal microorganisms, which feed on cellulose, and their physicochemical production parameters vary from species to species and to the kind of enzyme [15]. Major bacterial cellulase producers are *Pseudomonas* sp., *Escherichia coli*, *Serratia* sp., *Paenibacillus* sp., *Clostridium thermocellum*, *Bacillus subtilis*, and *Streptomyces* sp., with optimal temperature range between 30-50°C, and pH range between 4-6. These organisms have been reported to produce varied cellulases growing on numerous substrates ranging from agricultural residues, organic municipal waste, organic industrial effluents such as

sugarcane bagasse, corn cob, rice straw, rice husk, wheat bran, apple pomace, molasses, spent wash [14, 16]. Among fungi *Aspergillus niger* is well-studied for production of very high level of cellulase. Other species that have a significant contribution to cellulase (EC 3.2.1.X) production include fungi like *Penicillium*, *Trichoderma reesei*, and *Thermomyces lanuginosus*. These organisms also have the capability of utilizing various agricultural, industrial, and municipal waste for the production of a range of cellulase (EC 3.2.1.X) enzymes [17, 18]. Although numerous industrial cellulases are available, there is still a need for new enzymes with higher efficiency, yield, and higher stability, and for the same advanced techniques as genetic modification, immobilization, enhancing enzyme stability, and reusability through coculture systems are required [18, 19].

Hemicellulases (3.2.1.X) are a family of enzymes that hydrolyze hemicellulose, the complex polysaccharide of the plant cell wall; several types exist, depending on substrate specificity and mode of action [12]. These include endo-1,4- β -xylanases (EC 3.2.1.8) (hydrolyze internal β -1,4-glycosidic bonds in xylan); exo-1,4- β -xylanases (EC 3.2.1.8), also known as xylobiohydrolases (removal of the terminal xylose units from the xylan chain); β -xylosidases (releases free xylose from xylooligosaccharides and xylobiose); endo-1,4- β -mannanases (breaks the internal bonds of mannan) and β -mannosidases (hydrolyses mannoooligosaccharides into free mannose) [20, 21]. They find significant applications in food, paper, textile, etc. [22]. Hemicellulases are secreted by a broad range of microorganisms, such as bacteria like *Bacillus subtilis*, but mainly by fungi including industrial enzyme-producing *Trichoderma reesei*, which can secrete large amounts [23-25].

Lignases (EC 1.11.1.14) are essential enzymes that break lignin, a difficult-to-degrade and resistant polymer within the cell wall of the plant. These enzymes can be grouped into several types depending on their action mechanisms: laccases—multi-copper oxidases that catalyze the oxidation of phenolic compounds, and are involved in lignin polymerization; peroxidases, including lignin peroxidases (LiP) and manganese peroxidases (MnP), which use hydrogen peroxide to oxidize lignin and other aromatic compounds; and DyP-type peroxidases, known to oxidize a range of phenolic substrates, including lignin fragments [26]. Ligninases play fundamental roles in ecological and industrial contexts, facilitating lignin degradation, contributing toward the degradation of environmental pollutants like PAHs, and enhancing cellulose and hemicellulose availability for biofuel.

Recent research has underscored the origins, characteristics. It uses ligninases, with a particular focus on their generation by diverse fungal species, notably white-rot fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor*, both renowned for their lignin-degrading abilities [26]. Laccases and peroxidases are increasingly acknowledged for their industrial applications, encompassing textile bleaching, pulp and paper manufacturing, and bioremediation, attributable to their efficacy in oxidizing phenolic compounds [27, 28]. Progress in genetic engineering is under investigation to enhance the production and activity of ligninases, and research is focused on optimizing microbial strains to enhance lignin degradation.

Xylanases (EC 3.2.1.8) catalyze the hydrolysis of xylan (a significant component of plant cell walls composed of 23% lignin, 40% cellulose, and 33% hemicellulose). They can be differentiated into several classes based on modes of action [29]. The main groups are endo-1,4- β -xylanases, which cleave the internal β -1,4-glycosidic bonds of xylan polymers to give rise to shorter oligosaccharides; exo-1,4- β -xylanases, also called xylobiohydrolases, that release xylose or xylooligosaccharides from the non-reducing terminal parts of xylan chains; and β -xylosidases, responsible for the cleavage of xylooligosaccharides and xylobiose, releasing free xylose, thereby bringing termination to the

hydrolysis [30]. It also helps biotechnological applications, including biofuel and paper production [29].

Contemporary studies have underscored essential attributes of xylanases (EC 3.2.1.8), emphasizing their microbial origins, ideal environmental conditions, and a wide range of applications [31]. Xylanases (EC 3.2.1.8) are produced by various microbes, primarily fungi such as *Aspergillus oryzae* and bacteria when given temperature and pH ranges suitable for the industrial processes. Many xylanases are versatile bimetallic enzymes; whilst the fungal xylanases have optimum temperature and pH for activity at 30-50°C and pH 4.0-7.0 respectively, bacterial xylanases carry out their functions well between 40-70°C and at a pH of 6.0-9.0 [30]. Choosing the correct substrates is critical because it will lower the production costs and increase their application in large-scale industrial enzyme production. For example, wheat bran, corn cobs, rice husks, and barley husks lead to a much better usage and sustainable processes [29]. Improvements in the xylanase-based production have been made lately through genetic engineering, CRISPR-based strain improvement, and recombinant DNA technology of which aim at boosting desirable traits in xylanase, like thermostability and pH tolerance, in a cost-effective approach [5, 32-34].

Pectinases (EC 3.2.1.15) refer to enzymes that hydrolyse the structural polysaccharide pectin in the primary cell wall of plants [35]. They fall into various categories based on their mode of action. Main categories include polygalacturonases (PG), which break the α -1,4-glycosidic linkages in the backbone of pectin polygalacturonic acid, liberating oligomers of galacturonic acid; pectin lyases (PL), which catalyze the breakdown of pectin through a mechanism different than hydrolysis of glycosidic bonds, resulting in unsaturated derivatives; and pectin methylesterases (PME), which catalyze demethylation of pectin and affect its gelling properties [36]. Pectinases play essential roles in both ecological and industrial environments [37]. They break down pectin into simpler sugars, which are then utilized by the microbes, enhance food processing through clarification of fruit juices, improve the texture in products such as jam, and contribute to different applications in biotechnological fields such as textiles and paper.

Latest research has highlighted the source, optimum parameters, and applications of pectinases. The latter are produced by a variety of microorganisms, with most focus on the activity of pectinases by fungi, such as *Aspergillus* sp., and bacteria, including *Bacillus subtilis*, where fungal pectinases are the most advantageous in industrial usage due to their higher activities [38, 39]. Different pectinases have specific optimal temperatures and pH levels for their functional activity; some forms are more active at lower pH levels, which plays a crucial role in fruit processing. Advances in genetic engineering and synthetic biology are also being adapted to enhance the production and functional efficiency of pectinases in the microbial world, thereby engineering them for different industrial applications [Table 1].

Table 1 Major lignocellulolytic enzymes and technological advances employed for enhancing production.

Enzyme Type	Organisms Producing	Production Media (Physico-chemical)	Technological advances	References
Cellulases (Endoglucanases, Exoglucanases, β -glucosidases)	Bacteria: <i>Pseudomonas sp.</i> , <i>Escherichia sp.</i> , <i>Serratia sp.</i> , <i>Paenibacillus sp.</i> , <i>Aeromonas sp.</i> , <i>Clostridium thermocellum</i> , <i>Bacillus subtilis</i> , <i>Streptomyces sp.</i> , <i>Cellulomonas sp.</i> , etc. Fungi: <i>Aspergillus Niger</i> , <i>Penicillium sp.</i> , <i>Rhizopus sp.</i> , <i>Trichoderma Reesie</i> , <i>Beauveria sp.</i> , <i>Humicola sp.</i> , etc.	Temp: 30°C to 50°C, pH: 4-6; Media: agro-industrial wastes (apple pomace; kinnow waste; soybean hulls;) sugarcane bagasse; and rice, wheat, and corn cobs	Genetic engineering for hyper-production, Immobilization techniques for enhanced enzyme stability, Co-Culture System, Ionic Liquid Tolerance	[14, 40-42]
Hemicellulases (xylanases, endoxylanases, xylosidases, and arabinofuranosidases)	<i>Trichoderma</i> , <i>Hypocrea jecorina</i> , <i>Aspergillus</i> , <i>Penicillium</i> and <i>Talaromyces</i> , <i>T. emersonii</i> and <i>Talaromyces thermophilus</i> , <i>Penicillium decumbens</i> , <i>Talaromyces cellulolyticus</i> (previously <i>Acremonium cellulolyticus</i>) and <i>Talaromyces amestolkiae</i>	Temp: 28-45°C, pH: 5-7; Media: xylan, beechwood, malt extract, Wheat bran-based media	genomic and proteomic approaches, integration of extremophilic enzymes, submerged and solid-state fermentation	[43-46]
Ligninases (Lignin Peroxidase, Manganese Peroxidase, Laccase)	<i>Phanerochaete chrysosporium</i> , <i>Pleurotus ostreatus</i> , <i>Streptomyces</i> , <i>Trametes versicolor</i> .	Temp: 55-60°C (laccase), 30-55°C (peroxidases), pH: 3-6; Media: lignin, glucose, peptone	Metagenomics for discovering novel ligninases, Nanobiotechnology approaches for enzyme stabilization, transcriptomic and proteomic approaches	[47-49]
Xylanases	<i>Clostridium cellulovorans</i> , <i>Fibrobacter succinogenes</i> S85, <i>Thermoascus aurantiacus</i> , <i>bacillus subtilis</i> , <i>Aspergillus</i>	Temp: pH: 5-7.5;	CRISPR-Cas9 based strain engineering, directed evolution	[33, 50, 51]

	<i>niger</i> , <i>Trichoderma reesei</i> (<i>Hypocrea jecorina</i>), <i>Bacillus halodurans</i> C-125, etc.	Media: Xylan-based media (corn cob, rice husk), wheat bran	to improve thermal stability, and Metagenomic Approaches
Pectinases	<i>Aspergillus niger</i> CH4, <i>Penicillium frequentans</i> , <i>Bacillus polymyxa</i> , <i>Bacillus pumilis</i> , <i>Bacillus stearothermophilus</i>	Temp: 40-50°C, pH: 3.5-8.0; Media: Pectin-rich substrates (citrus peel, apple pomace), wheat bran, glucose	Industrial scale-up using solid-state fermentation (SSF), Extremophilic Fungi, genome-walking PCR, TAIL-PCR, and site-directed mutagenesis [52-55]

3. Applications of Lignocellulolytic Enzymes

Few significant applications of lignocellulolytic enzymes include their usage in food and beverage industries, pulp and paper industries, etc. and they have been listed in Table 2. Lignocellulolytic enzymes, notably cellulases and xylanases, have become integral to the food and beverage industry [9], expanding their utility beyond traditional biomass degradation. These biocatalysts, along with pectinases, are classified as food-macerating enzymes and have revolutionized fruit and vegetable juice production [56]. Their application in juice extraction and clarification processes has led to remarkable improvements, including higher yields, enhanced flavor profiles, and optimized viscosity [3, 9]. The paper industry has increasingly relied on agricultural and paper waste for production as virgin pulp supplies have diminished [32]. This transition involves various stages, including preparation, pulping, recovery, and bleaching [3]. Since the 1980s, lignocellulolytic enzymes have enhanced key processes like bio bleaching, drainage improvement, and effluent treatment [32]. These enzymes improve the quality, brightness, and overall appearance of the bleached pulp [3]. Ligninolytic enzymes also neutralize toxic chemicals in paper production [5]. Additionally, enzymatic hydrolysis has helped reduce energy consumption and improve selectivity, leading to an eco-friendly production process [3, 9].

Table 2 Applications of different types of lignocellulolytic enzymes.

Enzyme Type	Specific Applications	References
Cellulases (3.2.1.X)	- Biofuel production from lignocellulosic biomass - Textile industry (fabric softening and dyeing) - Pulp and paper industry (improving paper quality) - Food and beverage industry (enhancing the digestibility of feed) - Medical applications (wound debridement, tissue engineering)	[1, 3-5]
Hemicellulases (3.2.1.X)	- Food industry (improving texture, digestibility) - Pulp and paper industry (reducing lignin content) - Pharmaceutical industry (producing prebiotics) - Medical applications (treating digestive disorders) - Biofuel production from lignocellulosic biomass	[2, 5, 32]
Ligninases (EC 1.11.1.14)	- Pulp and paper industry (delignification) - Textile industry (fabric softening and dyeing) - Biofuel production from lignocellulosic biomass - Pharmaceutical industry (producing lignin derivatives) - Medical applications (treating cancers, inflammatory diseases)	[3, 7, 8]
Pectinases (EC 3.2.1.15)	- Food industry (clarifying fruit juices, improving texture) - Pharmaceutical industry (producing pectin-based drugs) - Medical applications (treating digestive disorders) - Textile industry (fabric softening and dyeing) - Bioremediation (breaking down pectin in waste materials)	[1, 2, 9]

Due to the scarcity of fossil fuel and the increasing demand for energy sources, it has become mandatory to look forward to alternative energy sources. Among all alternatives, second-generation bioethanol has been understood as a promising approach. It involves pretreatment and

saccharification of lignocellulosic biomasses and subsequent conversion to bioethanol through microbial fermentation [2].

This can combat climate change and lower greenhouse gas emissions [2]. Unlike first-generation biofuels, these advanced biofuels do not interfere with food crops [9].

Biorefineries utilize lignocellulosic biomass to produce various bio-based products through biochemical processes [32]. The primary goal is to convert renewable biomass into a spectrum of valuable chemicals, fuels, and materials, thereby replacing fossil fuel-derived products and contributing to sustainability. Lignocellulolytic enzymes play a crucial role in bioremediation by facilitating the biodegradation of lignin and complex organic compounds [5]. This capability aids in treating contaminated soil and water, making these enzymes valuable tools for environmental cleanup efforts by breaking down toxic substances. Production of biochemicals using lignocellulolytic enzymes finds tremendous applications [5]. These enzymes catalyze the conversion of complex polymers to simple sugars that can be converted to organic acid, solvents, and other platform chemicals. Enzymatic hydrolysis allows the production of organic acids (e.g., Lactic acid, succinic acid, etc.). These chemicals find applications in food preservation, pharmaceuticals, and biodegradable plastics [8]. Solvents like acetone, butanol, and ethanol are used in coatings, adhesives, and fuel additives [9]. Lignocellulosic biomass can be converted to furfural and hydroxymethylfurfural, which serve as intermediate compounds during the production of bioplastics, fibers, and pharmaceuticals [7].

Lignocellulolytic enzymes can also be widely used as animal feed [3] to enhance digestibility and nutritional values [5, 56]—improved digestibility results in better nutrient absorption leading to enhanced growth rates and overall health in animals. Livestock can derive more energy and nutrients from the feed, leading to better performance in terms of weight gain [1, 8], milk production, and reproductive efficiency, and is a cost-effective process [9].

Another promising application of these enzymes is the production of bioplastics through the breakdown of lignocellulolytic enzymes, thus helping develop more sustainable and eco-friendly material options.

These are a few prominent industrial applications of lignocellulolytic enzymes, which make them a sustainable solution and key market drivers.

4. Technological Advancements in Microbial Lignocellulolytic Enzyme Production

Recent strategies to increase cellulase (EC 3.2.1.X) activity therefore involve both developing better ways to ferment as well as genetic modification [19, 57]. Techniques like solid-state fermentation try to mimic the natural process of growth. In contrast, submerged fermentation is optimized in terms of parameters such as the type of substrate on which the enzyme is to be produced, pH, nutrient availability, temperature, and oxygen availability to get the highest yield of enzymes. More effectively produce chemicals by cheaper LCC substrates, such as agricultural residues and waste papers. Optimizing enzyme production through microbial strain improvement through random mutagenesis, genome shuffling, and gene cloning, higher levels of cellulase (EC 3.2.1.X) genes have been achieved through strong promoters as observed in *Trichoderma reesei*. The development of chimeric proteins and the application of RNA interference to silence repressive elements in the genome are also noteworthy advancements. Moreover, advancement in bioreactor technology enhances even distribution of temperature and moisture. It reduces the chances of

contamination, and using fed batch or continuous fermentation reduces repression from the accumulating reducing sugars. Leveraging metagenomics allows exploration of uncultivable microorganisms to discover novel cellulases, aiming to create robust strains that can function under extreme conditions and minimize downstream processing costs for industrial applications [Figure 1].

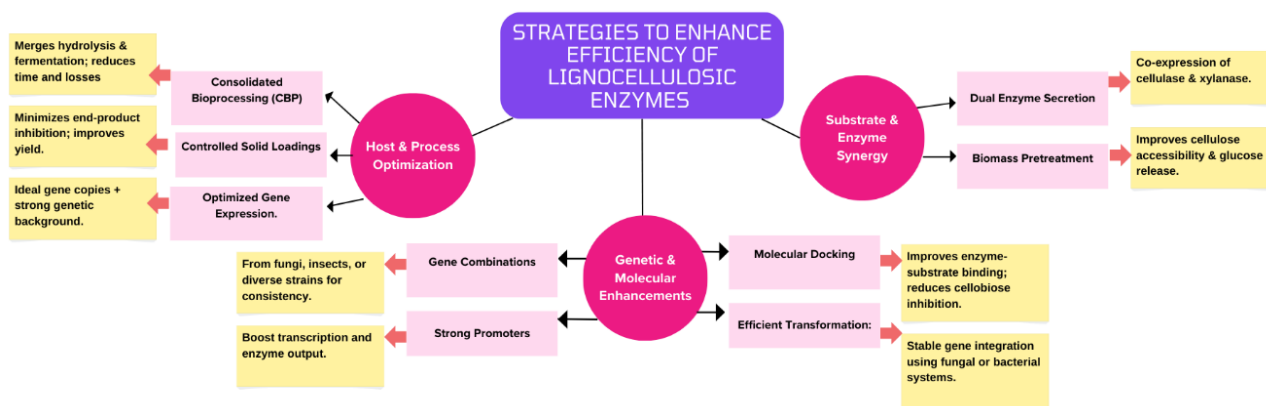


Figure 1 Proposed strategies to enhance efficiency of lignocellulosic enzymes.

Modern strategies and techniques for producing hemicellulase (3.2.1.X) have included genetic improvement and new bioprocessing methods [58]. Metabolic engineering and genetic mutation in strains under UV and gamma irradiation increase the productivity of enzymes in microorganisms like *Aspergillus* and *Trichoderma*. CBP (Consolidated bioprocessing) contains all three essential processes, i.e., enzyme production, hydrolysis, and fermentation. With the help of recombinant systems in *Saccharomyces cerevisiae* and *Escherichia coli*, the activity of hemicellulases (3.2.1.X) increases, especially thermostability. Higher yields are also achieved regarding co-cultivation of the microbial strain and bioreactor design, including Packed Bed and Rotary Drum. Efficiency is also improved in breaking down and recovering hemicellulose through acid hydrolysis, supercritical CO₂ technology, hydrothermal treatment, and alkali extraction [59]. Amalgamating these methods with ultrasonic and microwave-assisted extraction techniques escalates the efficiency to mass-produce hemicellulase (3.2.X.X) for its industrial applications in the biofuels, packaging, and biodegradable material industries [Table 3].

Table 3 Types of lignocellulolytic enzymes, their pilot scale production techniques, and process parameters.

Types of Enzymes	Major Techniques Involved	Physio-Chemical Parameters	References
Cellulases (EC 3.2.1.X)	- Solid-state fermentation (SSF) and submerged fermentation (SmF) - Genetic modification, RNA interference, and metagenomics	- Optimized pH (4-6), temperature (30-50°C), strain improvement - Nutrient availability, oxygen supply, fed-batch or continuous systems	[19, 57, 58]
Hemicellulases (3.2.1.X)	- Genetic engineering (UV and gamma mutation, recombinant systems) - Bioprocessing methods: consolidated bioprocessing (CBP), bioreactor design	- Optimized pH (5-7), temperature (28-45°C), and co-cultivation of strains - Packed bed, rotary drum bioreactors, acid hydrolysis, CO₂ technology	[58, 59]
Ligninases (EC 1.11.1.14)	- Genetic engineering, protein engineering, enzyme immobilization - Use of white-rot fungi (e.g., <i>Phanerochaete chrysosporium</i>, <i>Pleurotus</i>)	- Optimum pH (3-6), temperature (30-55°C), microbial co-culturing - Discovery of novel enzymes using metagenomics and synthetic biology	[60-62]
Xylanases (EC 3.2.1.8)	- Solid-state fermentation (SSF) for higher yields - Genetic engineering, CRISPR/Cas9, expression in <i>Pichia pastoris</i>	- Optimized pH (5-7.5), temperature (30-50°C), low-cost substrates - Wheat bran, corn cobs, rice husks, industrial process adaptation	[31, 63]
Pectinases 3.2.1.15	- Strain improvement, directed evolution, genetic engineering - Advanced recovery techniques (affinity chromatography, ATPS)	- Optimized pH (3.5-8), temperature (40-50°C), SSF, SmF - Fermentation using GRAS microorganisms (<i>Aspergillus niger</i>)	[35, 64]

Modern strategies for ligninase production focus on utilizing white rot fungi such as *Phanerochaete chrysosporium*, *Trametes villosa*, *Pleurotus ostreatus*, and marine derived fungi, such as *Mucor racemosus*, *Aspergillus sclerotiorum*, for production of lignolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP). Here, *Streptomyces viridosporus* and *Bacillus megaterium* are also considered to degrade lignin from bacterial sources. Recent improvements in discovery/functional genomic, transcriptomic, proteomic, metabolomic techniques, and synthetic biology have disclosed new lignin-degrading enzymes and developed bacterial strains with enhanced enzyme production [61]. Stability, scalability, and efficiency of enzymes have been improved over time through processes such as genetic engineering, protein engineering, enzyme immobilization, and co-culturing of microbes for uses in industries such as bio-fuels, paper industries, and bioremediation. These strategies have countered most drawbacks previously associated with fungal ligninases (EC 1.11.1.14) by leveraging the adaptability and resilience of bacterial systems [62].

Current approaches to improving xylanase production include optimum fermentation techniques where SSF (solid-state fermentation) is proven to provide higher yields and economic benefit over the submerged fermentation (SmF), even though using affordable and renewable substrates such as wheat bran, corn cobs, and rice husk [31]. Recent strategies in genetic engineering; recombinant DNA technology, and protein engineering are applied in enhancing xylanase yield, stability and enhancement of enzyme activity, bringing increased applicability of the enzymes in industries. Expression in other organisms, including *Pichia pastoris* and Filamentous fungi, including *Aspergillus oryzae*, has also been used to increase secretion, and to adapt the organisms to industrial levels. These approaches in addition to the proper choice of low-cost substrate and molecular tools like CRISPR/Cas9, have improved the xylanase production to suit the requirements of the various industries, including biofuel, animal feed, and paper industries. Adjusting conditions such as pH, temperature, and nutrient concentrations in the fermentation process has enhanced the yield and stability of the enzyme further [63].

The latest developments on pectinase production have focused on improving yield and effectiveness by employing novel techniques, including the use of the microbial source of *Aspergillus niger* due to its status as a GRAS and the presence of diverse enzyme genes [35]. This process SSF (solid-state fermentation) and SmF (submerged fermentation) were both optimized to achieve better results where SSF is preferred to be used for its high yield and low energy needs. Strain improvement and directed evolution, followed by advancement in the field of genetic engineering, have again contributed to increased production of enzymes [34, 64, 65]. Recent developments in enzymatic recovery and stabilization include affinity chromatography and aqueous two-phase systems (ATPS). These advancements benefit the increasing need for pectinases in the industrial sector, especially the food industry, textile and water treatment.

5. Market Demand and Conclusion

Due to increasing demands for sustainable alternatives and environmental solutions, market demands for lignocellulolytic enzymes are high as they are essential in bioconversion of lignocellulolytic biomass and are expected to constitute a significant portion of the global enzyme market, with a compound annual growth rate of 7.5% from 2024 to 2034. These enzymes represent

almost about 20% of the worldwide sales having significant applications in feed, food, textile, paper, biofuel, pharmaceutical, biomaterials and biopolymers, waste treatment etc.

Large-scale production optimization, specific activity, cost-effective pretreatment and operation processes, and increasing the shelf-life of biomolecules have always remained a significant challenge in their applications. There is always a requirement for more research and development to combat the pretreatment and operational costs and improve the efficiency and working capability of these enzymes. Thus, the global enzyme market, as well as the regional market for lignocellulolytic enzymes, is expected to grow high shortly.

The review has mentioned the usage of enzymes in various sectors, and it can be understood that there are multiple challenges faced by the enzyme-producing industries. Looking forward to the global increase in demand for enzymes, we would like to conclude that consistent and significant research on enzymes is needed at this time and will contribute effectively to the country's economy.

Author Contributions

Dr. Smriti Shrivastav has conceptualized the content and done overall alignment of content. Ms. Anjali Singh and Ms. Kashish Ujla have drafted the basic content. They have made tables and pictures for the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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