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An Insight Into Microbial Cellulases

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Abstract

Cellulase enzymes are a group of complex proteins produced by microorganisms that play a critical role in breaking down cellulose, a major component of plant cell walls, into simple sugars. The three main types of cellulase enzymes are exoglucanases, endoglucanases, and β-glucosidases, which work together in a sequence of interactions to efficiently break down cellulose into glucose molecules. This process is crucial for the recycling of cellulose in the natural environment and has great potential for industrial applications. One of the most promising industrial applications of cellulase enzymes is in the production of biofuels. Lignocellulosic materials, such as agricultural waste and forestry residues, can be used as a raw material for the production of biofuels. However, these materials are difficult to break down and require costly pre-treatment techniques to be converted into biofuels. Cellulase enzymes offer a potential low-cost alternative to these pre-treatment methods. Studies have shown that using cellulase enzymes to hydrolyze lignocellulosic materials can increase the yield and efficiency of biofuel production. Another promising industrial application of cellulase enzymes is in the paper and pulp industry. Cellulase enzymes can be used to improve the quality of pulp, increase paper brightness, and reduce the use of harmful chemicals in the production process. Additionally, cellulase enzymes have shown promise in textile cleaning and finishing, as they can be used to remove stains and improve the softness and color of fabrics. Recent advancements in biotechnology have led to the development of genetically modified microorganisms that can produce cellulase enzymes with improved properties and higher yields. Additionally, immobilization techniques have been developed to increase the stability and reusability of cellulase enzymes in industrial applications

Introduction

Enzymes are essential biocatalysts that are widely used in various industries, such as brewing, dairy, detergent, food and feed, pharmaceutical, and paper and pulp industries, for the production of goods used in daily life [1]. One of the most commonly used enzymes in these industries is cellulase, which is in high demand, according to current global cellulase market analysis reports. Cellulose, the primary constituent of plant materials, is the most abundant polysaccharide on earth and has been utilized for various purposes for centuries [2]. However, the practical applications of cellulose in the industrial sector have become more complex and diversified, making it an affordable and effective raw material for several industries [3]. This has led to a significant foundation for researchers to conduct transdisciplinary research on cellulose, including cellulose hydrolysis, which is catalyzed by cellulases. Cellulases are not a single enzyme but a group of enzymes consisting mainly of exoglucanases, including cellobiohydrolases and β-glucosidase [4]. In the natural environment, fungi, bacteria, and actinomycetes secrete cellulases that are either free or attached to the cell surface, which differ in their enzyme production efficiency and the composition of the enzyme complex [5]. *Trichoderma viride* and *T. reesei* are two of the most widely studied aerobic cellulolytic fungi, although both aerobic and anaerobic bacteria can produce these enzymes [6]. The enzyme dissolves β-1,4-linkages in the cellulose polymer to liberate sugar molecules such as glucose, depending on the industry's requirements.

According to recent market assessments, cellulase enzyme is increasingly used in various industrial sectors, including healthcare, textiles, pulp and paper, detergents, food, and beverages. The food and beverage industry, in particular, extensively utilizes cellulase in the production of fruit juice, wine, and coffee, while cleaning and washing agents, as well as laundry detergents, use it in other industrial applications. Moreover, cellulase is now recognized as a potent alternative to antibiotics for treating *Pseudomonas* biofilms, making it an excellent trend for addressing healthcare industry issues [7]. The use of microbes or microbial enzymes for the pre-treatment of lignocellulosic materials is currently gaining industry interest. The depletion of fossil fuel resources worldwide has led to an increasing focus on the generation of bioethanol from lignocellulosic biomass via enzymatic hydrolysis [8]. Lignocellulosic biomass is an inexpensive, easily accessible, and environmentally friendly raw material. However, it contains a tight network structure comprising cellulose, hemicellulose, and lignin, which creates challenges in its deconstruction [9]. Therefore, pre-treatment of lignocellulosic materials is critical in various industries, such as bioethanol production. The most effective pre-treatment method for lignocellulose is biological pre-treatment using cellulolytic bacteria and their enzymes, which make the crystalline structure of cellulose less rigid, allowing for the release of fermentable sugar forms [10].

However, the industry faces several significant challenges in using cellulase enzyme, including its lower catalytic effectiveness and higher cost. Another crucial factor is the poorly understood relationship between hydrolysis mechanisms and the molecular structure of the enzyme. To enhance the enzyme's catalytic activity further, it is crucial to have a better understanding of its composition and mechanism of action [11].

Therefore, the objective of this chapter is to discuss the composition and mechanism of action of cellulase and potential low-cost enzymatic pre-treatment techniques that have been used for lignocellulosic materials to make them an effective raw material for bioethanol production.

Cellulose

Before talking about cellulase, it is important to understand cellulose, the substance that the enzyme depends on. This section will provide a quick overview of cellulose.

Cellulose is a Polysaccharide

Cellulose is a linear polysaccharide composed of repeating units of β-D-glucose molecules linked by β-(1→4) glycosidic bonds. It has the chemical formula $(C_6H_{10}O_5)n$, where "n" denotes the degree of polymerization (DP) or the number of glucose units linked together. Cellulose chains contain long, unbranched sequences of anhydro glucose units (AGUs) with three free hydroxyl (-OH) groups at each glucose unit. The chains are held together by hydrogen bonds, both intra- and intermolecular, which create a highly ordered, crystalline structure [12]. The cellulose molecule is made up of both crystalline and amorphous regions, which are interwoven to create its complex structure. Cellulose has four main crystalline forms, labelled I, II, III, and IV, each with a distinct hydrogen-bonding pattern [13]. The strong intermolecular hydrogen bonding between the cellulose chains makes cellulose insoluble in water and most organic solvents.

Cellulose Organisation

Plant cell walls consist of several levels of structures, including single cellulose chains, elementary fibrils made up of tens of single cellulose chains, and microfibrils that are bundles of elementary fibrils. It is believed that the microfibril is made up of multiple elementary fibrils that have recently formed. As the cell grows, microfibrils split into separate microfibrils, which contain up to 40 cellulose chains and have a diameter of between 10 and 20 nm [14]. Microfibrils also contain non-cellulosic polymers such as hemicelluloses. Some researchers suggest that a microfibril is made up of several elementary fibrils, but this remains a topic of debate. The plant cell wall structure is supported by microfibrils, which also contain cellulose, and cross-links between the matrices of hemicellulose and pectin. Lignin, a complex polymer, typically fills the voids between the cellulose and pectin matrices and forms covalent connections with hemicellulose, mechanically strengthening the plant cell wall [15-16]. Other substances, such as lipids, polyphenols, resins, and minerals, are also present and classified as extractives in the lignocellulosic biomass structure found in plants.

Cellulase

Enzyme Demand Worldwide

Enzymes have become increasingly important in various industrial processes due to their wide range of applications. The global demand for enzymes has been identified to be driven by several significant factors, including economic advancements, such as the rising per capita income in developing nations. According to a market analysis by Freedonia published in January 2018, the demand for industrial enzymes is projected to increase at an annual rate of 4.0% to $5.0 billion in 2021. The rise in personal incomes in developing nations is also identified in this research as the primary driver of the growth in enzyme demand [17].

Scientific research on enzymes is mainly based on genetics, molecular biology, and biotechnology, and ongoing advancements in these fields, particularly those related to DNA sequencing and manipulation, have significantly increased the global need for enzymes. Among the enzymes with steadily rising demand is cellulase. The advancement of both basic and applied research on cellulase and its subsequent use in various industrial processes depend on the accumulation of knowledge about this enzyme [18].

Cellulase: Molecular Structure and Function

Cellulase enzymes are produced by a wide range of organisms, including fungi, bacteria, actinomycetes, protozoans, plants, and animals. The Carbohydrate-Active Enzymes (CAZy) database is a useful resource for information on glycoside hydrolase families, including cellulases, based on similarities in amino acid sequences and crystal structures. In total, the CAZy database has identified 115 glycoside hydrolase families. Additionally, there are 13 distinct families of cellulase genes that have been cloned and studied, with over 50 different 3D structures available [19].

Cellulases consist of catalytic modules and non-catalytic modules in their molecular structure. The catalytic modules are organized into various families based on their crystal structures and amino acid sequences, with some containing non-catalytic carbohydrate-binding modules (CBMs) and other known or unknown modules. The N- or C-terminus of the catalytic module may contain these non-catalytic modules. Bacteria and fungi produce cellulases that typically have two or more structural and functional domains, with the common arrangement being a combination of a catalytic domain and a cellulose-binding domain (CBD) in noncomplex cellulase systems [20]. The catalytic and cellulose-binding domains are considered the most important since they are involved in the enzyme's hydrolytic processes [21].

Cellulases catalyze the breakdown of cellulose polysaccharide by disintegrating β-1,4-glycosidic linkages [22]. The hydrolysis of cellulose microfibrils in the plant cell wall is mediated by three major types of enzymes: endoglucanase, exoglucanase, and β-glucosidase [23]. These enzymes work together to facilitate complete cellulose hydrolysis. Endoglucanase randomly attacks the internal bonds of loosely bound, amorphous regions of cellulose, producing new chain ends that can be easily attacked by other enzymes. Exoglucanase, on the other hand, targets the reducing or nonreducing ends of cellulose strands to produce glucose or cellobiose units. β-Glucosidase hydrolyzes cellobiose to glucose from the non-reducing ends.

Uses of Bacterial Cellulases

The use of cellulases has gained significant attention due to their wide range of applications in various industries. The food and beverage industry, textile industry, animal feed, and biofuels are among the main sectors that utilize cellulases. According to a report by Coherent Market Insights, the textile sector dominates the market for cellulases in 2017 [25]. The demand for cellulase was divided among the food and beverage industry, textile industry, and animal feed in 2016 [25].

Cellulases are used in various applications in the textile industry, including fabric processing, denim finishing, and bio-stoning. They are also employed in the paper and pulp industry for bleaching and in the laundry and detergent industry for stain removal. In the food and feed industry, cellulases are used for the production of fruit juice and wine, as well as in animal feed to improve digestibility [26]. Cellulases also have potential applications in the medical industry for wound healing and drug delivery [27]. The cellulase market is projected to reach $2.3 billion by 2025, with a compound annual growth rate of 5.5% from 2018 to 2025 [25].

Cellulases in Textile Industry

The textile industry has experienced significant growth in recent decades, with increasing consumer demand for unique styles, colors, and clothing driving its expansion. To meet this demand, the industry has embraced eco-friendly solutions, with cellulases being the third-largest group of enzymes used in textile processing. This has led to numerous industrial applications of cellulase enzymes, creating a competitive market platform.

Cellulases find wide use in wet processing applications, including bio stoning of denim fabric, biopolishing of textile fibres, softening of clothing, and removal of excess dye from fabrics. The fungal cellulase from *Trichoderma reesei* is the most commonly used enzyme in the textile industry [28]. Additionally, actinomycetes from the genera *Streptomyces* and *Thermobifida*, as well as other genera of bacteria such as *Pseudomonas* and *Sphingomonas*, are sources of enzymes employed for decolorization and degradation of textile dyes [29].

Bio stoning and biopolishing are two of the most widely recognized uses of cellulases in the textile industry. Bio stoning creates a worn-out look on denim fabric by treating it with cellulases, which break down the surface cellulose fibres [30]. Biopolishing, on the other hand, is a process used to improve the texture and appearance of textiles by removing protruding fibres and creating a smoother surface [31].

Cellulases play a critical role in the textile industry, providing eco-friendly solutions for achieving the desired appearance and texture of textiles. Through biostoning and biopolishing, cellulases are employed to create distinctive styles while reducing the industry's impact on the environment.

Bio-stone Washing

Denim washing typically involves three steps, including desizing, abrasion, and finishing. Traditionally, pumice stones have been used for the abrasion process. However, this method has several drawbacks, including reduced efficiency, increased wear on the machinery, and extensive back staining [32]. To address these issues, microbial cellulases have been developed as a substitute for pumice stones. Cellulases hydrolyze the cellulose fibre in the denim fabric, breaking down the 1,4-linkages of the cellulose chains and creating water-soluble sugars that remove the indigo dye, resulting in a faded appearance [32]. Studies have identified endoglucanase II obtained from *Trichoderma reesei* as a highly effective candidate for bio stoning, which has several benefits over stone washing with pumice stones, such as high productivity, a safer environment, reduced machine wear and tear, and quick treatment periods [33]. However, bio stoning also has its own issues, such as back staining. To address this, researchers have explored various biotechnological solutions, including immobilizing cellulases on pumice stones and using a mixture of amylase, cellulase, and laccase enzymes for bio stone washing [34].

Recent studies have also investigated the combination of xylanase from *Thermomonospora sp.* with an alkali-stable cellulase to reduce back staining tendencies [34]. However, it is crucial to neutralize the chemical by-products produced during dye removal, as they can be harmful to both humans and animals. Therefore, pre-treatment of the effluents produced by bio stone washing is necessary to eliminate any post-reaction intermediate chemical compounds that may contaminate soil and natural streams [32].

Biofinishing/Biopolishing

After several washings, cotton fabrics may become dull and fuzzy due to the growth of fibres on the fabric surface, resulting in the loss of the fabric's original appearance. This phenomenon is known as pilling. The biopolishing process aims to remove these microfibrils and improve the fabric's appearance, feel, and color vibrancy, as well as its hydrophilicity and moisture absorption [35].

T. reesei and *Aspergillus niger* are two fungi that produce acidic cellulases that are highly effective in biopolishing [36]. These enzymes break down the cellulose fibres on the fabric surface, resulting in a smoother and more polished appearance. This process not only improves the fabric's aesthetic but also has the added benefit of being environmentally friendly, as the enzymes used in biopolishing are biodegradable and harmless [37]. Biopolishing is a widely used process in the textile industry due to its numerous benefits. It is an effective way to improve the appearance and texture of cotton fabrics, making them softer and more comfortable to wear. It also helps to remove stains and dirt particles that may have accumulated in the fabric fibres, prolonging the life of the garment [38].

Bioscouring

Bioscouring is a process used to remove non-cellulosic impurities from cotton fibres using enzymes, primarily cellulases. Pectinase can also be used in combination with cellulases to break down pectin compounds present in cellulose fibres, which helps in breaking the unbroken bond between the cuticle and the cellulose fibre's main body, leading to the degradation of the primary cellulose wall of the fibre. This reaction results in the eventual degradation of the cuticle, making the fabric softer [39].

Biocarbonization

Biocarbonization is a process that utilizes enzymes to remove cellulosic or vegetal contaminants from wool fibres in a biological way. Even fabrics made of 100% cotton or cotton blends may contain a small amount of undesired cellulosic material, which can result in a lower quality finish and improper finishing. Traditionally, sulfuric acid was used for carbonization, but it was expensive, dangerous, corrosive, and risky. Enzymatic carbonization is a non-hazardous, non-corrosive, and environmentally benign process. This process involves the use of cellulases to remove vegetal contaminants from raw wool's surface. The addition of pectinases or other enzymes can enhance the effectiveness of the process. Enzymatic carbonization techniques have minimal impact on the fabric's color and hand feel, making them a more favorable option compared to sulfuric acid treatment [39] [40].

Lyocell Defibrillation

Lyocell is a biodegradable fabric made from treated wood pulp that is used in various products, such as clothing and automobiles. This material is produced through solvent spinning, with N-methylmorpholine N-oxide being commonly used as a solvent system [41]. Lyocell fibres have excellent properties, including softness, high absorbency, high strength in wet or dry conditions, and wrinkle resistance. However, a common issue with this material is the development of small, tangled fibrils on the fabric's surface, which is called fibrillation. Cellulases can be applied to lyocell to remove fibrils and improve its softness and appearance, while also reducing fuzz and pills [42]. Cellulase are known to alter the handle and drapeability and removed surface fuzz. Cellulase also reduced the tendency of fibres to pill and reduced fibrillation of lyocell. Cellulases have been applied for softening, defuzzing, depilling and pill prevention, improved drapeability and improved surface appearance after multiple launderings [43]. Therefore, enzymatic textile processing plants must install pre-treatment facilities and conduct water quality monitoring [44].

Paper and Pulp Sector

The pulp and paper industry is one of the largest industrial sectors globally, producing various products such as office and catalogue paper, glossy paper, tissue, and paper-based packaging [45]. According to the World Wildlife Fund (WWF), this industry uses more than 40% of all industrial wood traded worldwide.

The three biggest producers of paper globally are China, the United States, and Japan, which manufacture half of the world's total paper production [46]. However, Germany and the United States are the top importers and exporters of paper worldwide [47]. It is noteworthy that Americans consume the most paper globally.

The pulp and paper industry relies on renewable resources, including pulp and paper. As such, reusing and recycling are two widely used ideas in this business. Cellulases, which are enzymes produced by microorganisms, are typically applied for this purpose. Cellulases' use in this sector has become more widespread, with potential applications that have spread out into many other fields since the 1980s [48]. Examples of such applications include deinking, pulping, bioremediation of industrial wastes, bleaching, and fiber enrichment [49].

Pulping is a significant process in the paper and pulp industry. Refining and grinding woody raw materials for mechanical pulping leads to pulps with increased levels of particles, bulk, and stiffness. This process requires a lot of energy, making it an unprofitable choice for the industry. Meanwhile, bio pulping is an energy-efficient and environmentally benign process that uses cellulases and other enzymes. Studies show that bio pulping can lead to energy savings of about 20% to 40% (Manda & Nyanhongo, 2017). It produces little pulp particles as it refines the metal. During paper manufacturing, these particles slow down the drainage rate. Cellulases can easily break down these particles to improve the pulp's drainage capacity. Mixtures of cellulases (endoglucanases I and II) and hemicellulases have also been used for bio-modification of coarse pulp material to enhance fibre characteristics, making the hand sheets stronger [50]. Biological pulping has the potential to lower energy costs and have a smaller negative impact on the environment while improving the quality of pulp and paper products.

Deinking

Traditional deinking processes that use large amounts of chemicals are expensive, harmful to the environment, and can result in increased contamination. Additionally, the paper produced may not degrade properly. To overcome these drawbacks, cellulases and xylanase are used to deink different types of paper waste [52]. Partial hydrolysis of carbohydrate molecules releases ink from the fibre surface, a process that is carried out by cellulases alone or in combination with hemicellulases [53]. Enzymatic deinking offers several advantages over traditional methods, including improved brightness and a cleaner appearance of the paper, as well as environmental benefits [52].

Biomodification and Fibre Bio-characterization

The paper industry often simplifies the process of producing paper by improving the beat ability, runnability, and drainage of paper pulp. This can be achieved by altering the characteristics of fibres, which can reduce the need for alkali during the process. Cellulase and hemicellulase combinations have been successfully used to modify the attributes of fibres [54]. Enzymatic hydrolysis also aids in the characterization of fibres using various techniques, such as scanning electron microscopy (SEM) [55] and high-performance liquid chromatography (HPLC) [56].

Laundry and Detergent Industry

Enzymes have revolutionized the laundry and detergent industry, providing effective and eco-friendly solutions for stain removal and fabric care [57]. The use of enzymes in laundry detergents has been a common practice since the 1960s, and today it is estimated that the detergent sector accounts for around 25-30% of all enzyme sales, making it the largest single market for enzymes.

Cellulases are one of the most commonly used enzymes in laundry detergents due to their ability to remove stains and improve the texture and appearance of fabrics [57]. Cellulases are derived from various sources, including fungi such as *Trichoderma species (T. longibrachiatum, T. reesei, T. viride, and T. harzianum), Aspergillus niger, Humicola species (H. insolens and H. griseathermoidea),* and *Bacillus species.* In addition to cellulases, proteases, lipases, amylases, and mannanases are also commonly used in laundry detergents to improve stain removal and fabric care effectiveness [57]. For example, SaniZyme®, a liquid detergent containing four enzymes (lipase, cellulase, amylase, and protease), is used for cleaning surgical tools and endoscopic equipment of all sorts of blood, protein, mucus, fats, and carbohydrates. Similarly, Getinge Clean MIS Detergent®, a formulation that contains enzymes such as protease, lipase, amylase, and cellulase as well as surfactants, sequestering agents, and corrosion inhibitors, is designed to clean intricate, minimally invasive instrumentation. The global market for laundry detergents was estimated to be worth 133.3 billion USD in 2016, and the demand for enzymes in laundry detergents is expected to continue to grow due to the increasing focus on eco-friendly and sustainable products [58].

Agriculture

Cellulases have been utilized in agriculture for a variety of purposes, including controlling plant diseases and enhancing crop growth. Research has shown that specific fungal cellulases can break down the cell walls of plant pathogens [59]. Additionally, bacteria, such as plant growth-promoting rhizobacteria (PGPR), have been shown to play a significant role in promoting plant growth, suppressing potential plant infections, and protecting plants from the disease [60]. Certain fungi, including *Trichoderma sp., Geocladium sp., Chaetomium sp., and Penicillium sp.*, have been found to promote plant growth, hasten blooming, strengthen root systems, and boost crop yields [61]. While the precise mechanisms underlying these effects are not yet fully understood, it is believed that cellulase and other enzymes produced by these microorganisms may play a direct role [62]. In some cases, bacterial cellulase and antibiotic synthesis may work together to combat plant pathogenic fungi [63]. It has been observed that the addition of cellulosic materials, such as straw and *Gliricidia* leaves, to crops can improve soil quality and increase yield due to the additional nutrients provided [64]. Cellulolytic microorganisms are believed to contribute to this process. However, further research is needed to fully understand the mechanisms by which these microorganisms affect plant growth and disease control in agriculture. Thus the use of microbial cellulases in agriculture has great potential for improving crop yields and plant health. Further research is needed to fully understand the mechanisms involved and to optimize the use of these enzymes in agricultural practices.

Cellulases in Medicine

Medical pharmacology is a field that is constantly evolving, and cellulases are being explored for new medical applications. Although humans cannot produce cellulase, research has shown that consuming enzyme blends containing cellulase can be beneficial for human health. Cellulase derived from *Trichoderma reesei* and *Bacillus licheniformis* is widely used commercially to break down cellulose-rich foods, such as fruits, vegetables, cereals, legumes, grains, nuts, seeds, soy, dairy, leafy greens, sprouts, and herbs, as well as fats, sugars, proteins, carbs, and gluten. Some enzyme blends, such as VeganZyme®, are marketed as digestive aids for patients with metabolic diseases [65]. Beyond its use in enzyme blends, cellulase has potential medical applications. One indirect use of cellulases is the degradation of chitosan, a polysaccharide derived from chitin found in the exoskeletons of insects, shrimp, and other marine animals. Chitosan and its derivatives have been used in a wide range of medical applications, including artificial skin, surgical sutures, bone reconstruction, haemostatic dressings, anticancer and antidiabetic agents, hypocholesterolemic effectors, biopharmaceutics, and cosmetics. Studies have shown that cellulases can hydrolyze chitosan, and that chitosan treated with cellulase may have anticancer and antibacterial properties [66]. Cellulase can also be used directly for therapeutic purposes. For example, cellulase can be used to treat bezoars, which are obstructions in the digestive tract caused by ingested plant matter that cannot be digested. Cellulases from fungi have been used to treat bezoars, but the potential of bacterial cellulases for this purpose remains largely unexplored. Additionally, cellulases could be used to break down the cell walls of pathogenic organisms, such as Acanthamoeba, which can cause severe eye infections. Evidence suggests that Acanthamoeba cysts contain cellulose in their cell walls, making them susceptible to cellulase treatment. Similarly, biofilms formed by pathogenic microorganisms could be broken down by cellulases, potentially improving the efficacy of treatments for infections caused by these microorganisms [65]

Food Manufacturing Industry

The food manufacturing industry is a vital contributor to meeting the nutritional needs of the world's population. Enzymes such as cellulases play an important role in food biotechnology processes due to their wide range of applications, including juice clarification, modification of sensory properties of fruits and vegetables, extraction of compounds from plants, and improving the quality of bakery products [66]. One of the primary uses of cellulases in the food industry is in the clarification of fruit and vegetable juices. These juices often contain floating polysaccharides such as cellulose, hemicellulose, lignin, pectin, and starch that cause cloudiness. To address this issue, enzyme preparations containing cellulase, hemicellulases, and pectinases are used to clarify juice. For example, "Rapidase pomaliq," a commercially available enzyme preparation containing cellulase, hemicellulases, and pectinases from *Trichoderma reesei* and *Aspergillus niger*, is used to clarify fruit juice. Bacterial cellulases from *Bacillus* and *Paenibacillus* have also been found to work in conjunction with other enzymes to clarify fruit and vegetable juice [66]. In addition to juice clarification, enzymatic treatment using cellulase has been effective in reducing the viscosity and other rheological characteristics of nectars and purees, making them suitable for commercial use [67]. Cellulases and pectinases have also been found to be effective in modifying the sensory characteristics of fruits and vegetables. For example, cellulase is used to extract sugars from grapefruit peels that are utilized in the manufacture of food. Cellulase is also used in the extraction of phenolic compounds from grape pomace, a by-product of wine production [67] [69]. Cellulases play a significant role in the extraction of olive oil as well. Enzymatic treatment during the malaxing or mixing stage of the extraction process improves the extraction process and the quality of the olive oil. It increases the phenolic content and antioxidant activity of olive oil, leading to improved quality [68] (Bhatia et al., 2002). Enzyme cocktails that combine cellulases with other hydrolytic enzymes have been found to improve the quality of bakery products. For example, a combination of cellulases, hemicellulases, amylases, lipases, and phospholipases has been found to improve the flavor of dough, extend its shelf life, and increase volume after baking [67]. Cellulases are also widely used in the extraction of pigments from plants and plant-based products. Enzyme mixtures containing cellulase are used to treat fruit peels to extract carotenoids, which are used as natural food colorants [68].

Animal Feed Industry

Cellulases play a critical role in the animal feed industry by improving the digestibility of food based on cereals and enhancing the nutritional value of forages, resulting in higher-quality feed. Research has demonstrated the effectiveness of *Trichoderma* cellulase as a feed additive for improving the feed conversion ratio and the digestibility of cereal-based foods [70]. Ruminant forage feed is highly complex in composition, consisting of cellulose, hemicellulose, pectin, and lignin. Cellulase preparations, such as those derived from *Bacillus subtilis* bacteria, have been shown to improve fodder digestibility and increase the availability of absorbable nutrients. Enzyme mixtures containing cellulases and hemicellulases are commonly used for milk production, body weight gain, and nutritional enhancement in animal feed. Additionally, enzyme treatment of animal feed has been shown to remove antinutritional elements present in grains and other cellulosic materials. Improved fibre digestion and nutrient availability in ruminants' natural digestive processes are key benefits of using cellulases in animal feed. Partial hydrolysis of lignocellulose molecules by cellulases enhances the emulsification of food in the animal digestive tract, further increasing the availability of nutrients [71]. Overall, the use of cellulases in animal feed is a promising approach to improve animal health and productivity.

Biofuel Industry

The demand for energy in the modern world has led to an increase in the consumption of fossil fuels, which are non-renewable resources and their depletion is inevitable. As a result, there has been a growing interest in the use of biofuels, such as bioethanol, as an alternative energy source. Second-generation bioethanol production from lignocellulosic biomass has gained momentum due to its abundance and low cost. Lignocellulosic biomass is the most significant potential feedstock for the production of bioethanol and has an annual potential yield of 200 billion metric tonnes worldwide [72]. These feedstocks include agricultural waste, forestry waste, and other wastes. Lignocellulosic biomass is environmentally friendly and does not contribute to food insecurity since it uses inedible parts of plants. However, the production of bioethanol from lignocellulosic biomass is challenging due to the complex structure of the substrate, which includes lignin, cellulose, and hemicellulose. Pre-treatment procedures are required to make the substrate more accessible for the release of fermentable sugars. The pre-treatment process can be physical, chemical, or biological, with biological pre-treatment being a promising and environmentally friendly option [73] [74]. In recent years, there has been significant research on the use of biological pre-treatment methods using microorganisms such as fungi and bacteria. These microorganisms can secrete enzymes that break down lignocellulosic biomass, making it more accessible for subsequent hydrolysis and fermentation steps. Some of the most promising microorganisms for this purpose include white-rot fungi, brown-rot fungi, and cellulolytic bacteria [75]. Using lignocellulosic biomass as a feedstock for the production of bioethanol has the potential to offer a sustainable and eco-friendly energy source. The efficiency of the bioethanol production process can be increased and the environmental effect can be decreased by using biological pre-treatment techniques. It is anticipated that ongoing study in this area will significantly improve lignocellulosic biomass pre-treatment and bioethanol production. A complex mixture of cellulose, hemicellulose, lignin, and other substances makes up lignocellulosic biomass [76]. The lignin content of lignocellulosic biomass, which makes it challenging to hydrolyze cellulose and hemicellulose, is primarily responsible for its refractory nature. The use of microorganisms to break down lignin and increase the accessibility of cellulose and hemicellulose in biological pre-treatment is a viable method to solve this problem [77]. This method has the potential to greatly improve the yield of bioethanol from lignocellulosic biomass. The efficiency of biological pre-treatment utilising fungi, bacteria, and even insects has been reported in a number of investigations. Among the most often employed microorganisms for lignocellulose breakdown are white-rot fungi like *Phanerochaete chrysosporium* and *Pleurotus ostreatus* [78]. Investigations into the potential for brown-rot fungus to preferentially breakdown lignin include Postia placenta [76]. According to [77], lignin can be broken down by ligninolytic enzymes produced by bacteria like *Streptomyces* and *Bacillus*. In order to increase the effectiveness of lignocellulose breakdown, the use of genetically engineered microorganisms and mixed microbial cultures has also been researched [78]. Biological pre-treatment has a number of benefits over other pre-treatment techniques, including being less energy-intensive, creating less harmful by-products, and being more ecologically friendly [77]. To ensure the process is economically viable for large-scale bioethanol production, though, optimisation is needed. To further improve the effectiveness of biological pre-treatment for lignocellulosic biomass, research is now being done in this area with the goal of discovering novel microorganisms and perfecting the process conditions. Biological pre-treatment is a promising technique for the production of biofuels due to its low startup costs, low energy requirements, eco-friendly nature, and lack of inhibitor formation during the process. Additionally, it does not produce any harmful substances or toxic effluents that could harm the environment. However, the lengthy incubation period required for effective delignification is a major challenge in industrial-scale implementation. This slow hydrolysis rate of microbes, which is responsible for the delignification process, limits the application of biological pre-treatment . Another potential limitation is the potential consumption of fermentable sugars produced during pre-treatment by the same microbes responsible for delignification, which could decrease bioethanol yields.

To mitigate these challenges, cocultures or biofilms of effective ligninolytic microorganisms can be used, along with isolated fermentative yeast. White-rot basidiomycete fungi have been found to be more adaptable and effective in the process than other ligninolytic microorganisms. Some examples include *Ceriporiopsis subvermispora, Phlebia subseralis, Pleurotus ostreatus,* and *Lentinus edodes*, which manufacture ligninolytic enzyme systems such as lignin peroxidase, manganese peroxidase, and laccase [79]. Bacteria such as *Azospirillum lipoferum* and *Bacillus subtilis* have also contributed some laccases, although their capacity for lignin breakdown is lower than that of fungi [80]. Actinomycetes have been investigated for their role in lignin biodegradation, while *Penicillium camemberti* has shown potential for lignin breakdown in some studies. Solid-state fermentation is the preferred technique for biological delignification, and microorganisms can be grown directly on the feedstock during the process, or enzyme extracts can be used instead.

Further research is necessary to speed up the incubation period and perfect the delignification procedure, as well as to improve the effectiveness of microbial consortia for biological pre-treatment.

The need for effective biological pre-treatment procedures has become increasingly urgent, requiring improvements in enzymatic hydrolysis. To achieve this, it is important to identify high enzyme-producing species from the natural environment, choose the best strain and culture conditions, and find distinct microbial communities for biological preparation, also known as consortia. The use of biological pre-treatment is a crucial step in the production of biofuels and other bioproducts from lignocellulosic biomass. To improve the effectiveness of biological pre-treatment, researchers have focused on enhancing enzymatic hydrolysis, which involves breaking down the complex lignocellulosic structure into simpler sugars that can be fermented into biofuels. One approach is to identify high enzyme-producing species from the natural environment and optimize their culture conditions. Microbial consortia, which involve cooperative action among diverse bacteria and fungi, have also been found to be effective in lignocellulose degradation. In addition to selecting the right microbial species and culture conditions, process variables such as incubation temperature, incubation time, inoculum concentration, moisture, aeration, and pH conditions must be carefully tuned to achieve optimal results. Accessory enzymes, such as arabinases, lyases, pectinases, galactanases, and esterases, have also been shown to increase the effectiveness of hydrolysis. Several studies have investigated lignocellulose-degrading microbes isolated from the natural environment.

Conclusion

Cellulases have become a dominant force in the global market due to their extensive applications in various industries. Microbes have shown remarkable potential for producing cellulases, but researchers believe that there are still undiscovered species in the environment that can produce more effective enzymes. To meet the increasing demand for microbial cellulases, more research is needed to advance our understanding of these enzymes' underlying mechanisms. The development of new scientific fields such as molecular biology, microbiology, and biotechnology will aid in uncovering new and innovative methods to maximize the potential of cellulases. By making necessary adjustments to the current limitations, researchers can further unlock the untapped potential of microbial cellulases.

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