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Review

Cellulases: From Lignocellulosic Biomass to Improved Production

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Abstract: Cellulases are enzymes that are attracting worldwide attention because of their ability to degrade cellulose in the lignocellulosic biomass and transform it into highly demanded bioethanol. The enzymatic hydrolysis of cellulose by cellulases into fermentable sugars is a crucial step in biofuel production, given the complex structure of lignocellulose. Due to cellulases' unique ability to hydrolyze the very recaltricant nature of lignocellulosic biomass, the cellulase market demand is rapidly growing. Although cellulases have been used in industrial applications for decades, constant effort is being made in the field of enzyme innovation to develop cellulase mixtures/cocktails with improved performance. Given that the main producers of cellulases are of microbial origin, there is a constant need to isolate new microorganisms as potential producers of enzymes important for biofuel production. This review provides insight into current research on improving microbial cellulase production as well as the outlook for the cellulase market with commercial cellulase preparation involved in industrial bioethanol production.

Keywords: cellulase; lignocellulosic biomass; cellulase cocktails; cellulase market; bioethanol

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1. Introduction

Lignocellulose is currently the most abundant renewable biomass on the planet, representing a cheap and easily available raw material for the production of different biotechnologically important products [1]. Therefore, biofuels derived from lignocellulosic biomass have become a highly relevant topic in recent years because they offer a great alternative to fossil fuels. The extensive use of fossil fuels not only depletes their reserves on the planet but also contributes to environmental pollution [2]. Namely, the use of fossil fuels in production processes implies risky techniques, which often result in the creation of unwanted by-products and hazardous gases. In addition to being advantageous compared to fossil fuels, biofuels also help protect the environment by reducing greenhouse gas emissions [3,4]. As a result, biofuels have garnered global interest from both the scientific community and governments.

As the demand for alternative sources of renewable energy increases, cellulase enzymes are becoming increasingly important as efficient cellulose degraders. Cellulases have been used for more than thirty years for commercial purposes and occupy second place on the global market of industrial enzymes. Despite extensive research on cellulase characterization, they continue to be a focus of scientific and industrial research, emphasizing their crucial role in biofuel production [5,6].

Despite being one of the fastest-growing industries worldwide, the bioethanol industry still faces several challenges to be considered as a part of sustainable bioeconomies. One of the main issues that has to be assessed is related to discovering efficient microbial cellulase, for improving biomass conversion into simple sugars. To achieve this goal, researchers are developing sustainable enzyme production processes and upgrading existing procedures.

This review presents the latest research on cellulases, with an emphasis on the cellulase production process and recommended approaches for cost-effective hyperproduction of cellulases with improved catalytic properties for industrial viability.

2. Lignocellulosic Biomass

In recent decades, the increasing global demand for energy has made lignocellulosic biomass a focus of research due to its high potential as a renewable source for biofuel production. Lignocellulose represents a unique renewable source of carbon, from which different transformation processes can produce biofuels [7].

Lignocellulosic biomass is the plant material residue that is generated in abundant quantities worldwide. The 200 billion tons of lignocellulosic biomass are produced annually, which represents about 90% of plant material global production. On the other hand, 8–20 billion tons of lignocellulosic mass are considered available for utilization in the production of biotechnologically important products [8]. Considering that it includes about half the plant matter produced by photosynthesis, the lignocellulosic biomass is also called photomass [9,10]. Lignocellulose biomass includes agricultural waste (such as rice straw, wheat straw, corn straw, baggase, etc.) [11], forestry or wood waste [12], and cellulose waste (such as paper).

2.1. Lignocellulosic Biomass Sources

The sources of lignocellulosic biomass that can be utilized to produce biotechnologically important products are divided into two categories: current and traditional. However, there is now a growing emphasis on using modern resources with the goal of gradually replacing traditional energy sources. Lignocellulosic biomass is diverse, and the primary feedstocks used in biotechnological processes vary by geographic area [13]. There are numerous types of lignocellulosic feedstock based on their composition, structure, and origin [14]. Lignocellulosic biomass feedstocks include agricultural waste (such as rice straw, wheat straw, and corn cobs), energy crops, forest waste, organic fractions of municipal waste, cellulosic waste (such as paper), grass biomass, weeds, and aquatic plants. These feedstocks can be used in the biotechnological production of valuable goods such as biofuels and enzymes [14,15].

Agricultural residues and energy crops are significant sources of lignocellulosic biomass, as indicated by the volume of residues produced in global rice and wheat production [14]. Rice waste consists primarily of rice straw and husk [16]. Annually, 700–800 million tons of rice straw are produced, with Asia producing the majority of it [16]. Wheat straw is the most abundant lignocellulosic raw material in Europe and the globe, trailing only rice straw. It is estimated that approximately 350 million tons of wheat straw are globally available each year for biotechnological production [17]. In the United States and Brazil, corn waste and bagasse are key feedstocks for the bioethanol industries [14]. Corn crop residue consists of stalks, leaves, cobs, and husks. Bagasse, a dry, pulpy, fibrous substance that remains after crushing sugarcane or sorghum stalks is a large agricultural waste product of the sugarcane processing process, with an estimated global production of 317 to 380 million tons per year [12,14].

Additionally, forestry residue created during forest clearing, replanting, processing, and maintenance is considered to be one of the most significant sources of raw materials for the production of second-generation biofuels [12]. In China, agricultural and forestry waste account for 74% and 22% of bioenergy production, respectively [18]. Organic fractions of municipal and industrial waste should also be considered as potential sources of lignocellulosic materials for the production of bioethanol [13].

2.2. Lignocellulose Composition

Lignocellulose is one of the most prevalent biopolymers in nature and forms the structural basis of the plant cell wall. It primarily consists of two hydrocarbon polymers, cellulose and hemicellulose, and one phenolic polymer, lignin, which together make up

80% of its dry mass [19]. Additionally, lignocellulose contains small amounts of proteins, lipids, vitamins, and other compounds, such as cyclic hydrocarbons, ash, phenols, pectins, resin, and wax [9,10].

2.2.1. Cellulose

Cellulose is the primary component of lignocellulose, accounting for 30–50% of its dry matter [20]. It is a linear homopolymer of glucose with a crystalline structure, composed of 500–1400 glucopyranose monomeric subunits linked together by β-1,4 glycosidic bonds [10,21]. The repeating disaccharide unit of cellulose, cellobiose, consists of two glucose units that are oriented at an angle of 180°, enabling the formation of intramolecular hydrogen bonds [20]. Cellulose molecules are arranged in parallel and held together by hydrogen bonds to form microfibrils with a small diameter of 2–3 nm, which together make up the cellulose fibrils in the plant cell wall [21,22]. The core of the microfibrils is made up of cellulosic molecules, which have a regular three-dimensional crystal structure. In contrast, the periphery of the microfibrils has a paracrystalline structure, composed of irregularly distributed cellulosic molecules and inserted hemicellulosic molecules whose side chains are attached to the surface of the cellulosic microfibrils via hydrogen bonds [20,23]. The cellulose-hemicellulosic network is embedded in a hydrated gel matrix formed by pectins.

2.2.2. Hemicellulose

Hemicellulose, which contributes 20–35% of the dry matter in lignocellulose, is the second polysaccharide after cellulose [21]. This polymer is a branching polysaccharide composed of linked β-1,4 and β-1,3 glycosidic linkages between pentoses and hexoses (D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic, and D-glucuronic acids) [10]. Xylans, mannans, and galactans are the three categories into which hemicelluloses are categorized based on the monosaccharide that predominates in their structure. Because of chain branching and acetyl groups connected to the polymer chain, hemicellulose differs from cellulose in that it has a lower degree of polymerization, poorer physical strength, and an amorphous structure [21].

2.2.3. Lignin

Coniferyl, p-coumaril, and sinapyl are the three phenylpropanoid building blocks that make up lignin, a complex aromatic heteropolymer. Lignin makes up between 15 and 40 percent of lignocellulose and is a component of the plant's secondary cell wall, and it is characterized by an amorphous structure like hemicellulose [21]. This biopolymer binds to cellulose and hemicelluloses with covalent bonds and plays an important role in structural rigidity, impermeability to water (hydrophobicity), oxidative stress, and microbial assault resistance in plant cell walls [10,21].

2.3. Lignocellulose Recalcitrance

Recalcitrance is one of the most significant qualities of lignocellulose, which is related to the complex structure and composition of this biopolymer and limits its hydrolysis [24]. Studies suggest that the physical and chemical properties of cellulose, hemicellulose, and lignin are responsible for recalcitrance, although the mechanism underlying this phenomenon is not yet fully understood [21,24]. Physical characteristics such as cellulose crystallinity, accessible surface area, degree of polymerization, particle size, and pore volume, and chemical characteristics such as lignin and hemicellulose content and composition, as well as the presence of acetyl groups, all have an effect on recalcitrance [21]. The available surface area is an essential factor that influences the hydrolysis of lignocellulose. This property is affected by two other physical characteristics of lignocellulose: porosity and particle size. A high degree of porosity and small particles result in a large accessible surface area, which enables more efficient lignocellulosic hydrolysis and a high yield of simple sugars. Thus, before enzymatic hydrolysis, lignocellulose is mechanically pulverized to a specific particle size that is specific for each lignocellulosic material. In the case of

porosity, the pore size is the most important factor, which must be larger than hydrolytic enzymes to allow their penetration into lignocellulosic biomass [21].

Enzymatic hydrolysis is also influenced by the crystallinity and degree of polymerization of cellulose. The cellulose crystallinity represents the relative abundance of crystalline cellulosic regions, in which the enzymatic hydrolysis is even 30 times less than in the amorphous regions of this biopolymer [21]. In addition, changes in the crystalline cellulosic regions affect the degree of polymerization of this molecule. Due to the presence of more hydrogen bonds and longer cellulose chains, the cellulose crystallinity is higher and the hydrolysis of lignocellulose is limited.

However, the reduced degree of polymerization of hemicelluloses has no impact on enzymatic hydrolysis, but rather the presence of complex side groups, as well as the interactions between these two polysaccharides and lignin, limits the accessibility of the enzymes [21].

3. Cellulases—The Cellulose Degrading Enzymes

Enzymes that break down lignocellulose are essential for the depolymerization of lignocellulose biomass. Apart from hemicellulases and ligninases, which degrade hemicellulose and lignin, cellulases are responsible for the bioconversion of cellulose to glucose.

In this bioconversion, cellulases face great challenges due to the insoluble and often recalcitrant nature of cellulose. Given that glucose, as a product of cellulose degradation, is further used to produce biofuels, cellulases play a fundamental role in cellulose hydrolysis.

Cellulases hydrolyze the β-glycosidic bonds in the carbohydrate molecule [25]. For efficient cellulose hydrolysis, a synergistic action of the cellulase enzyme complex consisting of three major types of enzymes—endoglucanase (endo β-1,4-D glucan glucanohydrolase, EC 3.2.1.4); exoglucanase or cellobiohydrolase (β-1,4-D-glucan cellobiohydrolase, EC 3.2.1.91); and β-glucosidase or cellobiase (β-D-glucoside glucohydrolase, EC3.2.1.21)—is required [26,27].

Endoglucanase degrades cellulose by cleaving the β-1,4 linkages within the cellulose chain at amorphous sites, releasing glucose, cellobiose, and other oligosaccharides. While endoglucanase acts only on amorphous sites of the cellulose chain, exoglucanase is responsible for the degradation of the crystalline parts of cellulose. Exoglucanase or cellobiohydrolases act from the end of the chain and split the long-chain oligosaccharides (produced by the action of endoglucanase) to cellobiose. Cellobiohydrolases can function as type I or type II, depending on whether they act from the reducing or non-reducing portions of the cellulose chain. Endoglucanase is also known as CMCase (carboxymethyl cellulose—carboxymethylcellulose), while exoglucanase is also known as Avicelase (avicel—microcrystalline cellulose) [28]. β-glucosidase or cellobiase hydrolyzes cellobiose and short-chain oligosaccharides producing glucose [25,29]. Since it participates in the final step of hydrolysis where cellobiose is converted to glucose, β-glucosidase is the enzyme that limits the rate of degradation [30]. Glucosidases are used in combination with other cellulolytic enzymes to produce glucose efficiently during the generation of biofuel from lignocellulosic biomass [25]. In addition to cellulases, GH61, initially classified as glycoside hydrolases family 61, offers enormous promise in breaking down the crystalline structure of cellulose [31]. Many ascomycetes and basidiomycetes (white and brown rot) fungi secrete GH61 proteins (or lytic polysaccharide monooxygenases—LPM Os), which are important in the breakdown of lignocellulose. The expression of most enzyme-encoded genes increases rapidly during fungal growth on lignocellulosic substrates, indicating that GH61 enyzme is involved in cellulose breakdown [31]. The GH61 enzymes are copper-dependent oxidases that breakdown crystalline cellulose oxidatively in a Cu- and reductant-dependent manner, greatly contributing to the complete degradation of the recalcitrant lignocellulosic substrate [31,32]. Because cellulose is surrounded by a network of hemicellulose and lignin, hemicellulases and ligninases are also required enzymes for effective lignocellulose biomass degradation.

Given the complexity and heterogeneity of the hemicellulose structure, the degradation process of this polymer generally requires the action of multiple hemicellulases. Since the xylan is the largest component of the hemicellulose, endoxylanase (endo-β-1,4-xylanase, EC 3.2.1.8) and xylosidases (1,4-β-D-xylanxylohydrolase, EC 3.2.1.37) are the main degrading enzymes of the xylooligosaccharides. β-mannanase (endo-1,4-beta-mannanase, EC 3.2.1.78) hydrolyzes hemicelluloses composed mainly of mannans and releases short mannooligomers, which can be hydrolyzed, releasing mannose by β-mannosidases. Other hemicellulases, which belong to debranching enzymes, are α-L-arabinofuranosidase, α-D-glucuronidase, acetylxylan esterase, feruloyl esterase, and *p*-coumaryl esterase [29]. For the degradation of lignin, four main ligninolytic enzymes—laccase (EC 1.10.3.2), lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), and versatile peroxidase (EC 1.11.1.16)—are used [33].

4. Production of Microbial Cellulases

Both bacteria and fungi have been extensively researched for their ability to produce cellulase. These cellulolytic microorganisms mostly degrade carbohydrates and cannot utilize lipids and proteins as a source of energy for metabolism and growth [34].

Literature reports are inconsistent when declaring the preference of bacterial cellulase regarding cellulase of a fungal origin. Some researchers revealed that bacterial cellulase is much more advantageous than the fungal one, with regard to facilitated mass transfer, increased specific activity, and improved stability [5]. There are a large number of cellulolytic bacterial strains that produce specific, highly resistant enzymes to extreme surrounding conditions [35]. Among the bacteria that are characterized by exceptional resistance to extreme temperature values, and those that produce cellulases, are *Pseudomonas* sp., *Bacillus* sp., *Escherichia* sp., *Serratia* sp. [36–39], *Bacillus* sp. [39–41], *Paenibacillus* sp., *Aeromonas* sp. [28,37,40,42], *Clostridium* sp. [43], *Streptomyces* sp. [44,45], and *Cellulomonas* sp. [46,47]. These bacteria are usually isolated from the environment under extreme climate conditions [5,48].

On the other hand, a large number of cellulase are produced from fungi, mostly from *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Trichoderma* sp., *Beauveria* sp., *Humicola* sp., etc., [8,34,49–54]. Fungal cellulases consist of nearly 70% (*w*/*w*) of cellobiohydrolases, accompanied by endoglucanases (20% *w*/*w*), and a small amount of hemicellulases [55]. However, compared to bacterial cellulase systems, fungal cellulases are structurally less complicated. Bacterial and fungal cellulases are often made up of two or more functional and structural domains that are connected together by a peptide linker. Fungal cellulases typically feature two different domains: the cellulose binding module (CBM) and the catalytic domain (CD), which are linked to the CBM by a short polylinker region at the N-terminus [56,57]. The catalytic binding domain of fungal cellulases is composed of less than 40 amino acid residues, three of which are conserved aromatic residues [57]. Bacterial cellulases, on the other hand, develop faster and have more genetic diversity than fungal cellulases, which are more widely available commercially.

There is a great advantage of fungi in terms of its ability to produce cellulolytic enzymes during their growth on low-cost substrates, in comparison to bacteria [49]. It is particularly beneficial that the cellulases from fungi may be obtained by simplified extraction methods [35]. Additionally, since they produce a whole range of cellulases, filamentous fungi remain the most popular for the industrial production of cellulases. The focus of examination is mostly on *Trichoderma* sp., whereby *T. resei* is the highly employed strain [58]. In this relation, in the following, the described aspects regarding cellulase production will be referred as cellulase of fungal origin.

4.1. Factors Affecting Cellulase Production

Fermentation method, carbon and nitrogen source, pH, temperature, salt and metal ions concentration, time, aerations, and fungal species are a few of the fermentation

parameters that fundamentally affect the synthesis of cellulases [35,49]. Some of the factors affecting cellulase production are discussed below.

4.1.1. Solid State Fermentation vs. Submerged Fermentation

There are two main methods for cultivating fungal species to produce cellulases: solid state fermentation (SSF) and submerged fermentation (SmF). In SSF, the fungi are grown on individual or several substrates with low moisture, gradually exploiting the solid substrate over a period of several days, which allows for prolonged growth [49]. SSF has been applied (at laboratory scale) to obtain cellulase from various fungi, such as *Lichtheimia romosa*, *Phaffomycetaceae*, *Dipodascaceae*, *Trichoderma citrinoviride* AUKAR04, and *Humicola insolens* MTCC 1433, using different types of carbohydrate substrates [49,59]. It is revealed that SSF is used only to a limited extent industrially, although continued attempts for efficient bioreactor designs at a large scale are being made [59].

On the contrary, in submerged fermentation (SmF), the fungi are grown in a free-flowing liquid that has been supplied with various nutrients. The growth of fungi is morphologically identified by pellet production, which contains fungal mycelia and is furthermore affected by the agitation speed [59]. As the medium nutrients and supplements are quickly consumed, a constant supply is required [49]. SmF has been used to derive cellulases from various fungi, including *Trichoderma reesei* and *Aspergillus niger*, in the first line, but so many others as well [49]. Table 1 presents the fermentation parameters, microorganisms used in SmF and SSF, as well as obtained cellulse activities.

However, since the enzymes are secreted into the liquid culturing medium in SmF, they may become greatly diluted, requiring additional steps to concentrate the enzyme solution [59]. With regard to the competence of the SSF in relation to SmF for cellulase production by fungi, Table 2 presents the summary of the strengths and weaknesses of both fermentation techniques.

Table 1. Review of fermentation parameters, microorganisms used in SmF and SSF, as well as obtained cellulase activities.

Microorganism	Process	Substrate	Fermentation Conditions	Cellulase Activity	Reference
Trichoderma reesei QM9414	SmF	Pea hulls	91 h, 30 °C, pH 5	Filter paper activity 0.372 ± 0.019 U/mL	[60]
Aspergillus niger ITV 02	SmF	Delignified sweet sorghum bagasse (10% *w*/*v*), 0.9 g/L urea, 2.4 g/L ammonium sulfate, and 1.5 g/L yeast extrac	50 h, 30 °C	Endoglucanase activity 0.61 ± 0.025 Endoglucanase specific activity 126.72 ± 1.83 β-glucosidase ativity 0.41 ± 0.006 Specific β-glucosidase ativity 85.0 ± 0.40	[61]
Bacillus aerius MG597041	SmF	Yeast extract of 0.5 g/L, peptone of 0.5 g/L, FeSO4 of 0.2 g/L, and K2HPO4 of 0.02 g/L	24 h, pH 5.5, 37 °C	Filter paper activity 127.4 IU/mL/min	[62]
Bacillus subtilis K-18	SmF	Potato peel	50 °C for 24 h of fermentation period; 2% substrate concentration, 2% inoculum size, 1% yeast extract, and pH 5.0,	3.50 ± 0.11 IU/mL	[63]
Hymenobacter sp. CKS3	SmF	5.0% corn stover, 2.5% molasses	4 days	CMCase 1.11 IU/mL Avicelase 0.92 IU/mL	[64]
Trichoderma stromaticum AM7	SSF	Peach-palm waste	12 days 26 °C	CMCase 120 U/g	[65]
Aspergillus niger NRRL3	SSF	Soybean hulls	96 h 30 °C	Endoglucanase activity 5914.29 U/L Exoglucanase activity 4551.19 U/L β-glucosidase activity 984.01 U/L	[66]

Table 1. *Cont.*

Microorganism	Process	Substrate	Fermentation Conditions	Cellulase Activity	Reference
Streptomyces fulvissimus CKS7	SSF	Rye bran	6 days 30 °C Solid moisture ratio 1:1	Endoglucanase (CMCase) 8.62± 0.08; Exoglucanase (Avicelase) 5.98 ± 0.22	[45]
Sinorhizobium meliloti 224	SSF	Waste tobacco	2 days 28 °C	Avicelase activity 1.503 U/g Carboxymethyl cellulase activity of 1.615 U/g	[67]
Penicillium oxalicum EU2106	SSF	Cassava residue	5 days 28 °C	34.0 ± 2.8 filter-paper units/g dry substrate	[68]
Bacillus subtilis MS 54	SSF	Maize bran	3 days 37 °C	CMCase 28.84 IU/g)	[69]
Talaromyces verruculosus IIPC 324	SSF	Wheat bran	4 days 24 °C Moisture content 62.5%	Endoglucanase 250 U/g	[70]
Fomes fomentarius TMF2	SSF	Sunfower meal	6 days 30 °C Moisture content 62.5%	CMCase 1.49 ± 0.10 U/g Avicelase 1.02 ± 0.07 U/g	[71]
Schizophyllum commune TMF3	SSF	Sunfower meal	6 days 30 °C Moisture content 62.5%	CMCase 2.51 ± 0.13 U/g Avicelase 1.60 ± 0.09 U/g	[71]
Bjerkandera adusta TMF1	SSF	Brewer's spent grain	6 days 30 °C Moisture content 62.5%	CMCase 2.76 ± 0.16 U/g Avicelase 2.76 ± 0.16 U/g	[71]

Table 2. Strengths and weaknesses of SSF and SmF during cellulase production by fungi.

	SSF	SmF
Strengths	The high productivity Utilization of alternative low-cost substrate Minimal power required Minimal water production No or little foam up creation Displays the native habitat of filamentous fungi, which is why they are better adjusted Generating greater enzyme volumes that can be isolated or used for direct, extraction-free hydrolysis of biomass	Sterilization, heating, and mass transfer are simplified Enhanced process tracking (temperature, pH, and soluble molecules), and automation Simple access to extracellular secreted enzymes Large commercial facilities use SmF for cellulase synthesis since there are advanced bioreactors available, which allow simple mass transfer and easy processing
Weaknesses	Heat generation Lack of knowledge of automation Slow aeration and reduced mass transfer Possible high level of mycotoxins	Continuous supply with medium supplements or nutrients is needed Low-end product purity necessitates additional refining

4.1.2. Nutrients

When cultivated on media with a carbon supply composed of plant polymers or short oligosaccharides, fungi release extracellular cellulase enzymes. However, the expression of these enzymes is suppressed when the cells are grown on medium containing sugars that can be quickly metabolized, such as glucose [49].

Moreover, the nitrogen source present in the fermentation medium stimulates the formation of cellulase [72]. The fermentation medium used to produce cellulase may contain various nitrogen sources. The available organic nitrogen sources include peptone, yeast, tryptone, soybean meal, and beef extract [49]. Inorganic nitrogen sources such as ammonium chloride, ammonium sulfate, and ammonium hydrogen phosphate may also be used [49]. It has been speculated that different nitrogen sources may induce distinct cellulase components [72].

Furthermore, the phosphorus source and trace elements (such as Zn, Ni, Mg, and Cu), supplemented in a fermentation medium, may also act in concert with the induction of cellulase [72].

4.1.3. Temperature

Temperature optimization is crucial for enzyme production since it directly affects microbial growth and protein secretion. This parameter should be adjusted based on the microorganism used and the overall process performance. High temperatures during SSF can negatively impact cellulase generation due to enzyme denaturation. Therefore, an ideal fermentation temperature should balance fungal growth and cellulase synthesis [72]. Most commonly used fungi, such as *Penicillium* sp. and *Aspergillus* sp., showed optimal cellulase production at temperatures ranging from 30 °C to 50 °C [50,73–75], while *Trichoderma* spp. have optimal temperatures between 30 °C and 40 °C [56,57].

4.1.4. pH

pH is another important parameter that affects enzyme production and fungal growth; however, monitoring pH, especially via SSF, is generally difficult. Therefore, this parameter is usually adjusted at the beginning of the process without further control. Since the pH fluctuations during fermentation were so small, they were typically ignored. If modest changes do occur, they are generally caused by the fungi's metabolic processes [72]. The highest activity of Avicelase and β-glucosidase produced by *Acremonium cellulolyticus* was reported at a pH range of 5.5–6, while the highest CMCase activity was observed in a pH 4 environment. In addition, the culture with a pH of 6.0 showed the highest cellulase activity among cultures with pH ranging from 3.5 to 6.5 [76]. As reported in the literature, optimal cellulase production by *Penicillium* sp. [73] and *Aspergillus* sp. [77] is attained at pH 4.0–5.0. In the case of *Trichoderma* spp., the optimal pH for cellulase production is between 4 and 6 depending on the microorganism strain [56,57].

4.1.5. Incubation Time

Incubation time is highly related to microorganism growth dynamics. When most fungi are grown on a lignocellulosic substrate under optimal conditions, they start to grow by spreading the tips of their hyphae. Usually, the prime indication of fungal growth was reported on the second day of SSF, within the greatest amount of research on the synthesis of cellulase [72]. Extracellular enzymes are generated during the colonization stage of fungal growth and are intended to break down the lignocellulosic substrate into smaller soluble molecules that might be further used as stimulants for growth. Depending on the overall process conditions, different fermentation times may be needed for the individual cellulase components to reach their peak activity.

As mentioned before and with regard to the literature reports, the incubation time for cellulase production by most of the employed fungi may highly vary, from 2 days to 16 days [50,52,72,74,78,79].

4.1.6. The Content of Moisture

In SSF, the substrate's moisture level is a crucial parameter to investigate, particularly because it is directly linked to the structure of the lignocellulosic substrate. The porosity and specific surface area of the solid particles control air diffusion productivity and water holding capacity [72]. When the moisture content is below the appropriate level, the solubility of nutrients is constrained, which prevents fungi from efficiently absorbing them. However, if the moisture limit is exceeded, a significant amount of water will surround the substrate particles, causing them to clump together and reduce air diffusion between the particles and the environment. Moreover, applying too much moisture to the SSF increases the risk of contamination [72].

Moisture content below 60% and above 80% is generally unfavorable for both cellulase production and fungal growth in SSF, regardless of the type of fungi [72].

4.2. Approaches for Cost-Effective Cellulase and Bioethanol Production

Even though extensive studies has been carried out on characterizing cellulases from various fungi, due to their widespread applications, further research should concentrated

on the improvement of the production process, particularly with regard to the cost reduction and thermostability of the enzyme, as well as the construction of strains for hyper cellulase production, which should be viable on an industrial scale. For instance, because they increase the reaction rate, diffusion coefficient, and solubility of the substrate while lowering viscosity, contamination, and cost, thermostable cellulases are widely preferred by the bioprocessing industry [80].

The main current and future potential of cellulase application is highlighted in the production of bioethanol. However, cellulase is a substantial cost contributor to the bioethanol production process [81]. This is the driving force behind amplified global interest among researchers for making cellulase production costs as low as possible, and it contributes to bioethanol production in sustainable bioeconomies.

For achieving this goal, several approaches have been proposed, and they are related to the strain improvement by metabolic engineering to increase the stability and specific activity of the enzyme, using low-cost raw materials as a medium for enzyme production, taking advantage of new developments in bioreactors in the manufacturing process, and implementing integrated enzyme production technology. The major prospects that need to be considered with regard to economic enzymes and bioethanol production are discussed below.

4.2.1. Strain Improvement and Genetic Engineering

The production of affordable cellulases has undergone dramatic advancement through genetic manipulation. However, the cultivation of either *T. reesei* or *A. niger* led to a lack of a specific cellulase component, despite the fact that fungi are capable of producing a complete cellulase system. For instance, *T. reesei* is unable to produce significant amounts of β-glucosidase, while *A. niger*'s cellulase system is deficient in endoglucanase and exoglucanase [72].

To create improved enzyme producers, genetic engineering tools, promoter design and selection, and metabolic engineering with native or synthetic transcription factors, other techniques and tools are employed [58]. Understanding the physiology of cellulase producers is crucial since it helps in the process of finding the target gene for alteration [81]. The use of genetic modification and bioengineering as cutting-edge methods for comprehending processes and creating strains for lignocellulosic bioconversion has received attention [58,82,83]. For instance, Häkkinen et al. (2014) [48] successfully identified multiple putative regulatory genes and demonstrated that the overexpression of these genes in *T. reesei* had a substantially beneficial impact on the production of cellulase and hemicellulase.

Given that the gene for cellulase expression is tightly controlled by transcription regulatory network in *T. reesei*, engineering transcription factors can improve the entire cellulosic system [84]. At least five transcription activators, four transcription repressors, and transcription factors that control cellulase biosynthesis have all been connected to the modulation of cellulase gene expression so far. In *T. reesei*, three distinct strong promoters (Pegl2, Pcbh1, and Pcdna1) control the expression of the transcriptional activator xir1. The overexpression of this transcription activator increases cellulase production significantly and enhances the level of additional proteins involved in the breakdown of lignocellulose [84]. In *A. niger,* two endoglucanase-encoding genes, eglA and eglB, are controlled by the xylanolytic transcriptional activator XlnR. When *A. niger* is grown on d-xylose, four cellulolytic genes as well as the xylanolytic genes are expressed, indicating a single regulatory mechanism that controls the transcription of all of these genes [85].

Furthermore, researchers have reported several mutagenic agents that positively affect various strains of fungi such as ultraviolet (UV) irradiation, gamma irradiation (Co60 x-rays), ethyl-methane sulphonate (EMS), and N-methyl-N'-nitro-N-nitrosoguanidine [35,86].

4.2.2. Waste Substrates as Medium Constituents for the Synthesis of Enzymes

Cellulase is commonly produced using commercial cellulose as a carbon source. However, to reduce production costs, alternative substrates, such as agro-industrial waste, have been explored. This approach involves using lignocellulosic biomass or waste as a replacement for the carbon source in the original medium. It is important to use a lignocellulosic substrate that contains enough nutrients to support fungal growth [72].

Several agro-industrial wastes, including apple pomace; kinnow waste; soybean hulls; sugarcane bagasse; and rice, wheat, and corn cobs, among many others, have been used as substrates for the economically efficient production of cellulases [52,71,72,74,87]. These wastes are typically categorized based on their woody and herbaceous nature. Moreover, multiple researchers have discovered that a fungal strain can create distinct isoforms of cellulase, and these isoforms exhibit varied behavior while interacting with the same substrate [88]. Moreover, using lignocellulosic biomass to produce enzymes addresses the issues of waste management and environmental damage brought on by the dumping of this waste.

The use of agro-industrial lignocellulosic waste in the synthesis of cellulases and other microbial enzymes, on the other hand, can result in a reduced enzyme yield due to the limited availability of cellulose due to the presence of the stiff polymer lignin and its microcrystalline structure. Prior to the cultivation procedure of the selected microorganism, agro-industrial lignocellulosic waste is exposed to physical or chemical pretreatments to disorganize the plant's cell wall structure, eliminate lignin, and improve microbial growth and enzyme production [89,90]. Different pretreatments, whether physical or chemical, have a different effect on the production, yield, and price of the produced enzyme. Chemical pretreatments are found to be superior to other methods. Although numerous standard physical and chemical procedures have been utilized to date for the pretreatment of these materials, with promising results, some secondary contamination has been discovered, reducing their usefulness in the process of microbial growth and synthesis [90].

4.2.3. Integrated Cellulase Production

Off-site, on-site, and integrated cellulase production are the three current configuration options used for the manufacture of cellulose [58].

Typically, traditional cellulase production technology is off-site, which means that cellulase must be transported and formulated with stabilizers due to the need for storage once it is purchased or produced in distant facilities [58].

In an on-site arrangement, cellulase manufacturing takes place at a nearby application facility, such as a bioethanol plant. This avoids the need for transportation and formulation, and it is therefore more cost-effective than an off-site configuration [58]. The on-site synthesis of cellulase will be less expensive because shipping and stabilization processes can be skipped, saving money on those expenses. Additionally, it has been demonstrated that on-site processes release significantly fewer greenhouse emissions than the use of purchased enzyme [58,59,88].

An integrated strategy is defined when the cellulase production facility in an on-site design employs less expensive pre-treated cellulosic biomass as substrate, which may be the same pre-treated biomass as that utilized for ethanol production [58].This design often ignores the need for extremely complex down streaming processes and avoids transporting and storing enzymes [58]. So, compared to the previous two specified configurations, an integrated arrangement is even more cost-effective.

Consolidated bioprocessing, which operates all of the processes in a single vessel, is another intriguing option in addition to these three. Saccharification and ethanol conversion are carried out in a single bioreactor by either a single microbe or a consortium [50]. However, this strategy requires a lot of future clarification and the resolution of its own problems.

4.2.4. Co-Culture System

Mixed cultivation is the growing of two or more microorganisms simultaneously under similar environmental circumstances, simulating a natural ecosystem [81]. This novel strategy boosts enzyme production and completes the cellulase system by accounting for genomic differences among fungal species [59]. Hence, the primary problem in a mixed culture is compatibility between the participating microorganisms, which can be solved by the cooperative interactions of the included microorganisms.

Mixed culture has a lot of advantages, including greater substrate usage, improved environmental adaptability, and increased yield and productivity [81]. By raising the level of resistance during the fermentation process, mixed culture lowers the likelihood of contamination by any undesirable microbial development [81]. Another benefit of mixed cultivation over monoculture is the production of all of the cellulases needed for the hydrolysis of biomass [81]. Thus, the co-cultivation of fungus may be a particularly effective method for generating enzymes locally for use in cellulosic bioenergy and biorefinery processes [35,59,81].

4.2.5. Improvement of Bioreactor Design

By solving the current difficulties, which are mostly related to fungal development, bioreactors with suitable designs should increase cellulase production. As agitation and aeration become more challenging due to fungus fermentation, heat and mass transmission are reduced and become more common problem on a big scale [58,72].

It is possible that sufficient aeration and mixing will solve the mass transfer issue, but it is also possible that the moist lignocellulosic substrate particles will combine and grow to produce larger particles [58,72]. Moreover, the depolymerization of a lignocellulosic substrate during fungal growth can be prevented by adding some inert materials to the substrate bed, which will stop the shrinkage that results from the mass transfer issue [72]. Additionally, it was proposed that nanobubble technology may hold promise for addressing aeration problems and mass transfer because a nanoscale air bubble would not be disturbed upon reaching the medium's surface and would remain hung there until used [58].

On the other hand, the heat-transfer issue may be resolved by supplying aeration to the substrate bed or by misting water onto it to encourage the evaporative cooling mechanism in eliminating excess heat [18]. Moreover, cooling plates can effectively remove surplus metabolic heat that is created within the substrate bed [72].

4.2.6. Cellulase Immobilization

Enzymes perform better when immobilized in the commercial sector, especially in terms of stability and reusability, resulting in more effective catalytic processes and, ultimately, lower costs [5].

Research has shown that immobilized cellulase exhibits better structural stability, maintains high activity for a long time, and remains active at high pH compared to the free enzyme [5]. The immobilized cellulases can also significantly enhance their thermal stability and have a higher affinity for substrates [5,80].

Cross-linking, copolymerization, fiber ultra-filtration, aqueous two-phase systems, and the usage of a wide range of both natural and synthetic supports can all be used to efficiently immobilize cellulase [5,87,91,92]. Even on an industrial scale, the cross-linked cellulase aggregates constitute a useful form of immobilized enzyme for usage in large-scale production procedures and biotransformation [93]. As a result, glutaraldehyde is commonly used as a cross-linking agent because it is inexpensive and readily accessible in terms of production and consumption [93].

In contrast, the type of enzyme support chosen is determined by the nature of the enzyme, its substrate, and its application [91]. Chemical stability, physical toughness, and price are important properties of an enzyme carrier [94]. Several materials, including nanoparticles, functionalized multiwall carbon nanotubes, copolymers, silicate clay minerals, modified chitosan beads, and modified activated carbon, were used by researchers as

carriers for the immobilization of cellulose [94]. Another crucial approach for immobilization is the entrapment of an enzyme in calcium alginate, which is known to be a quick, safe, and affordable procedure [91].

4.3. Cellulase Market Outlook

The estimation of the worldwide cellulase market in 2022 is USD 1621 million. By 2032, the market is anticipated to have grown at a CAGR of 6.9%, or roughly USD 3153.1 million [95,96]. The growing use of cellulase is witnessed in various industries, includingthe textile, pharmaceutical, cosmetic, pulp and paper, agricultural, and bioethanol industries. The biofuel industry has grown rapidly in recent years, with Brazil, France, and the United States leading the way. According to market research, this industry will generate more than USD 950 million by 2024 [88]. As a result, the growing demand for enzymes is closely related to the growth of bioethanol production, and it is growing faster than the other enzyme application industries. Due to these circumstances, cellulase is the second most important carbohydrase used in biofuels, trailing only amylases [88]. Particulary in the pulp and paper industry, the adoption of cellulase has increased; it requires reduced energy while refining and assists in enhanching paper quality [97,98]. Cellulase also improves freeness, cleaness, and brightness, while deinking the paper, and it eliminates the use of alkalis.

Moreover, the growing demand for cellulase in alcoholic and non-alcoholic beverages owing to the improved yield and quality of beverages is among the other factors projected to drive the growth of the cellulase market in years to come [97]. The demand for cellulase is growing in the agricultural sector, to improve the fertility of the soil, to improve plant growth, and to prevent and control diseases in plants. Additionally, the rising production of wheat and other cereals is propelling the future growth of cellulase.

Geographically, the global cellulase market includes North America, Europe, Asia Pacific, the Middle East and Africa, and Latin America. During the forecast period, Asia Pacific is also expected to provide gainful opportunities in this market. Factors that are contributing significantly to higher revenue generation in this region, particularly in India and China, include rising demand for cellulase for pharmaceutical products and packaging, printed books, clothing, and essential goods, as well as rising disposable income and a rising overall standard of living. Furthermore, Asia Pacific has seen significant growth in the textile industry as a result of growing consumer preference for clothes that fit well. Other developing regions are also expected to see significant growth in this market [95,96].

In the global cellulase market, North America accounts for a 34.4% market share. Among all of the other regions, North America is predicted to lead the market owing to the growing production and increased use of biofuel. In the recent past, the demand for biofuel has been soaring, and therefore the production too has increased; this factor has increased the potential of cellulases. According to https://www.futuremarketinsights.com/reports/cellulase-market (accessed on 7 March 2023) [99], the largest application of cellulase is in animal feed, followed by food and beverages, textiles, and the biofuel industry.

The top players in the cellulase market include Novozymes (Bagsvaerd, Denmark), Genencor (DuPont, Rochester, NY, USA) (Palo Alto, CA, USA), DSM (Heerlen, Netherlands), AB Enzymes (Darmstadt, Germany), Amano Enzyme (Nagoya, Japan), Primalco Ltd. (Tampere, Finland), BIO-CAT (Keswick, VA, USA), Zhongrong Technology Corporation Ltd. (Tangshan City, Hebei, China), Shandong Longda Bio-Products Co. Ltd. (Yishui Country, Shandong, China), and Sunson Industry Group (Beijing, China) [99,100]. Just a small number of industries sell their product formulations in the public domain, but the majority conceal or never disclose the formulations of their enzyme combinations and sell them under exclusive licenses. These businesses are actively involved in R&D, with the goal of offering their customers better, more technologically advanced products. To meet the rising demand for these products from farmers and consumers, several of them regularly introduce new types of products that contain cellulase enzymes.

4.4. Commercial Cellulase for Bioethanol Production

Biofuels derived from the most abundant biomass—lignocellulosic biomass—are a great alternative to fossil fuels. They play an important role in environmental protection by reducing greenhouse gas emissions [3]. The increasing demand for an alternative source of renewable energy leads to increased interest in cellulases as efficient cellulose degraders. One of the greatest challenges in the bioethanol industry is discovering a highly efficient cellulase preparation that can improve biomass conversion into simple sugars [101,102].

Cellulosic ethanol or second-generation bioethanol is ethanol produced from the waste lignocellulosic biomass. Compared to the first ethanol generation, which is produced from food and feed crops, the substrate lignocellulose for second-generation bioethanol is abundant and does not compete directly with food production [3]. Production of the second generation of ethanol is costlier than the first generation of ethanol due to the very complex network of cellulose, hemicellulose, and lignin, which needs to be degraded. Additionally, the whole process is more complex and requires expensive material and industrial inputs [88].

Since cellulases have wide applications in the biofuel industry, there are many industries involved in cellulase production. The main goal of all industrial companies is the development of an inexpensive and effective cellulolytic enzyme cocktail for efficient lignocellulose degradation. These "cellulase cocktails" are not composed of one type of cellulase but rather consist of several types of cellulases, including hemicellulases and β-glucanases. Globally, Novozymes and Genencor (DuPont) are the two leading companies specializing in commercial cellulase production [27].

Novozymes is a Danish company and today certainly the world's largest manufacturer of industrial enzymes. This company produced a family of cellulase preparations/blends called Cellic®. Based on twenty years of cooperation with the cellulosic industry, Novozyme has developed the technology for freeing cellulose and hemicellulose from lignocellulosic biomass, lowering the total cost of ethanol production [103].

The largest cellulosic ethanol plants in Europewas launched in 2013 in Crescentino, Italy. This cellulosic plant uses Novozymes' Cellic® technology for lignocellulosic degradation and the production of cellulosic ethanol [104]. The Novozymes Cellic® enzyme complex has the ability to hydrolyze a variety of lignocellulosic biomass materials into simple sugars, including municipal trash and agricultural residues, including wheat and rice straw, bagasse, corn stover, and cobs. Some energy crops such as miscanthus and bamboo are also lignocellulosic biomass [103]. Apart from cost reduction, other benefits of using Cellic® are a reduction in hydrolysis time, a reduction in the severity of pre-treatment of lignocellulose, increasing the total solid loading while decreasing the enzyme (Cellic®) dosing, and increasing the biomass-to-sugar conversion [103].

Cellic®CTec presents a mixture of cellulases, a combination of cellobiohydrolase and β-glucosidase with the presence of endoglucanase [105]. Over time, Novozymes launched improved versions of previously reported Cellic®CTec. The first improved version of Cellic®CTec was named Cellic®CTec 2 and Cellic®HTec 2. For these cellulases, Novozymes stated that they are "state-of-the-art" enzymes for the degradation of cellulose to fermentable sugars. At the time, when it was first launched in 2010, Cellic CTec2 was five times better at biomass degradation than other enzymes available on the market [106]. Cellic®CTec 2 are aggressive cellulases with a high level of β-glucosidase and with the addition of hemicellulase, while Cellic®HTec 2 are endoglucanase with a great affinity for soluble hemicellulose. A suitable combination of Cellic®CTec 2 and Cellic®HTec 2 could enhance cellulose hydrolysis if the pre-treated feedstock or lignocellulosic biomass has a significant amount of hemicellulose. Both enzyme mixtures are stable, highly concentrated, and effective at high solid concentrations, which is confirmed by published research studies [43,107,108]. Additionally, Cellic®CTec 2 and Cellic®HTec 2 are inhibitor-tolerant and compatible with multiple feedstocks and pretreatments. Although the optimal temperature and pH for Cellic® CTec2 are 45–50 °C and pH 5.0–5.5, the lower temperatures typical of simultaneous saccharification and fermentation (SSF) are ideal for this cocktail's

performance [103,109,110]. A literature survey showed that commercial cellulase preparation Cellic® was successfully used for SSF on 32 °C [111], 25 °C [112], and 30 °C [113] of various lignocellulosic substrates for bioethanol production. A novel member of a Cellic family, Cellic® CTec3, was introduced as the next generation of enzymatic innovation in the field of cellulosic biofuels. This enzyme mix consists of enhanced cellulase and hemicellulase. Cellic® CTec3 exceeds Cellic® CTec2 by 1.5 times and allows for a more economical conversion of biomass to ethanol. When compared to other used enzymes, biofuel producers using Cellic® CTec3 only require one-fifth of the enzyme dose. Novozymes claims that Cellic®CTec 3 is very efficient and that just 50 kg of the combination is required to produce 1 ton of ethanol from biomass. Comparatively, to produce the same amount of ethanol, a rival enzyme product must weigh at least 250 kg [114].

Cellic® CTec3 contains cellulase components augmented by proprietary enzyme activities, such as advanced LPM Os compounds, improved β-glucosidases, and a new set of hemicellulase activities, all of which work together to improve conversion efficiency [115]. Cellic® CTec3 HS is a highly efficient cellulase and hemicellulase complex with proprietary enzyme activities that boost the blend's cellulases. Advanced AA9 molecules and improved β-glucosidases are responsible for very high Cellic® CTec3 HS activity. It is estimated that the efficiency of the Cellic® CTec3 HS, compared to Cellic® CTec2 HS, is 1.5 times higher [116]. The advantages of the Cellic® CTec3 HS include a significant decrease in ethanol production costs overall, excellent process and substrate adaptability, and new options to enhance conversion procedure (increasing the biomass to sugar conversion, lowering the enzyme dose, raising the total solids loading, speeding up the hydrolysis process, and lessening the pretreatment's severity) [117]. Sun and coworkers [118] tested Novozymes Cellic® preparations, CTec1, CTec2, and CT3, for the hydrolysis of pre-treated lignocellulosic hydrolysis. With the same enzyme loading, CTec3 resulted in maximal glucan hydrolysis of pretereated poplar and steam-pre-treated sweet sorghum bagasse, while CTec2 and CTeC1 showed lower hydrolysis efficiency under the same experimental conditions. The obtained results showed that this cellulase preparation was tailored gradually in terms of the variety and proportion of enzyme proteins in order to achieve a better hydrolysis performance on lignocellulosic biomass. Additionally, further experiments showed that a large number of β-glucosidase, cellobiohydrolase I, and especially β- xylosidase were added in the CTec3 preparation. In addition, when some leading accessory enzymes such as endoxylanase, β-xylosidase, and AA9 (auxiliary activities family 9, formerly known as GH61; LPMOs -lytic polysaccharide monooxygenases) were directly added into the CTec1 cellulase, hydrolysis was increased significantly. With increased AA9 addition, the hydrolytic performance of CTec1 improved greatly. At an addition of 2.0 mg of AA9, the enzymatic hydrolysis of steam pre-treated sweet sorghum bagasse was up to 26% for 24 h and at 39% for 48 h, which was almost equivalent to that with the Htec addition at 2.0 mg. The results indicated that the AA9 addition exerted a positive role in improving the CTec1 activity [118].

Further improvement in Cellic technology was reached in 2015 as customized Cellic® 1.0, through the customization of enzymes to match specific processes and feedstock. New customized Cellic® solutions and further process optimizations are obtained to reduce biomass conversion costs. The key benefits of customized Cellic® solutions compared to Cellic® CTec3 are yield boost, robust activity, contamination control, and lower operating costs. Customized Cellic® solutions are made for improved hydrolysis performance on xylan-rich substrates; broader pH and temperature profiles provide robust activity, higher hydrolysis temperature, and lower pH and inhibit contamination. In addition, the percent of biomass conversion into soluble sugars is higher using customized Cellic® solutions rather than Cellic® CTec3 [104].

In terms of industrial biotechnology and enzyme innovation, Genencor has long been at the top of the world rankings. Now a part of DuPont, it provides enzyme products for biomass hydrolysis and offers complete solutions along the entire biorefinery value chain. Genencor, a Danisco A/S division, launched a commercially available family of

cellulolytic enzymes for the improved hydrolysis of lignocellulosic biomass for cellulosic ethanol production, named Accellerase® (Figure 1).

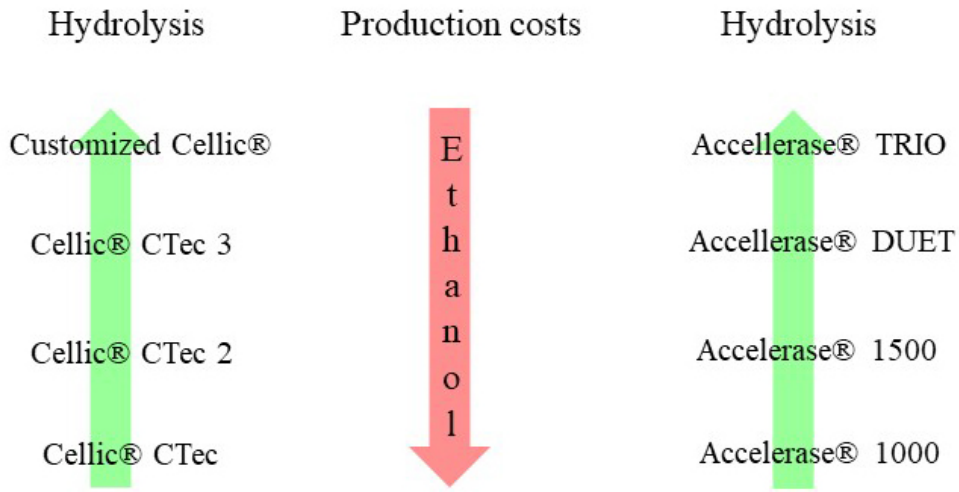

Figure 1. Improved hydrolysis of lignocellulosic biomass using Cellic® family and Accelerase® family, while decreasing the production costs of cellulosic ethanol.

The first generation of these powerful enzymes was Accelerase® 1000. Soon after, Accelerase® 1500 was designed to replace Accelerase® 1000, due to its lower cost operation and higher β-glucosidase activity for more complete biomass conversion to glucose [117]. Accelerase® 1500 is produced with a genetically modified strain of *Trichoderma reesei* and contains multiple enzyme activities: exoglucanase, endoglucanase, hemicellulase, β-glucosidase, and others [119–121].

When the Accellerase® DUET enzyme complex was introduced on the market, this technology was a breakthrough in enzyme hydrolysis from Genencor [122]. This enzyme complex converted cellulosic biomass into fermentable C5 and C6 sugars at a three times lower dose than Accellerase® 1500. Exoglucanase, endoglucanase, β-glucosidase, and hemicellulases, including xylanase, are the major enzymes present in the Accellerase® DUET enzyme complex, along with flexible feedstock. This enzyme complex can hydrolyze a variety of feedstocks, including paper pulp, corn stover, corn fiber, corn cob, wheat straw, wood chips, waste paper, municipal solid waste (MSW), and energy crops such as switch grass. Many pretreatment feedstocks, such as those that have been treated with diluted acid, ammonia fiber expansion (AFEX), and steam expansion, can be used with Accellerase® DUET. Simultaneous saccharification and fermentation (SSF), sequential hydrolysis and fermentation (SHF) in two steps, or hybrid saccharification and fermentation (HSF) arrangements can all be used to accomplish this [123,124].

Improved enzyme complex Accellerase® TRIO offers a two-fold dose reduction compared to Accellerase® DUET and reduces the cost of ethanol production. This product possesses engineered β-glucosidase for increased glucan conversion and reduced product inhibition [123].

Meanwhile, Genencor also launched three new Accelerase® enzyme products for small-scale process development that enhance biomass hydrolysis and ethanol production. These enzyme blends are Accellerase® XY accessory xylanase enzyme complex [125], Accellerase® XC accessory xylanase/cellulase enzyme complex [126], and Accellerase® BG accessory β-glucosidase enzyme [127,128]. In order to obtain high glucan conversion for the lignocellulosic biomass processing sector, Accellerase® BG β-glucosidase is created as an auxiliary product to augment entire cellulases low in β-glucosidase. In the hydrolysis of numerous feedstocks, including paper pulp, corn stover and cob, sugarcane bagasse, wheat straw, wood chips, waste paper, and many others obtained from various pre-treatment technologies such as diluted acid, ammonia fiber-expansion (AFEX), and steam expansion,

Accellerase® BG enzyme can be used in conjunction with whole cellulases. Simultaneous saccharification and fermentation (SSF), two-step sequential hydrolysis and fermentation (SHF), and hybrid saccharification and fermentation (HSF) combinations are all possible with the employment of the Accellerase® BG enzyme [123].

5. Conclusions

The literature reports revealed that incredible work has been carried out on the production and characterization of cellulases from both bacterial and fungal origins, owing to their widespread applications in many industries. While most research is still based on a laboratory-scale, there are many attempts to make industrial production of cellulases more viable. Fungal cellulase is predominant in industrial production, with the main focus on *Trichoderma* sp. The global cellulase market is likely to expand in the forecasted 10-year period, with a projected compound annual growth rate (CAGR) of 6.9% from 2022 to 2032, due to the widespread usage of cellulase in many industries, including agriculture, paper and pulp, and bioethanol. Although the bioprocess has experienced remarkable growth and success in addition to overall cellulase production, there is still a long way to go before ethanol from biomass can be produced at a cost that is competitive with liquid petroleum fuels. The high cost associated with processing cellulase is one of the major reasons that could limit the growth of the cellulase market.

Many leading companies involved in tailored cellulase production are focused on improving cellulase characteristics and finding solutions for efficient biomass hydrolysis. Their main goal is to produce cellulase or cellulase mix, with enhanced characteristics, for improved hydrolysis while lowering the production costs.

Several approaches have been proposed to achieve this goal, including strain improvement by metabolic engineering for improved enzyme stability and specific activity and tolerance, using less expensive raw materials as a substrate for enzyme production, new advancements in the production process bioeractors, and the adoption of integrated enzyme production technology.

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