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Ligninases and Glycosyl Hydrolases in Developing a cellulosic Bioethanol Industry

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Abstract: The lingocellulosic wastes are produced by industries, forestry, agriculture and municipalities. The accumulation of these wastes result in several environmental problems, health issues and safety hazards. These lingocellulosic wastes are economically attractive materials for cellulosic bioethanol production because of the large amount of potential sugar for fermentation and bioenergy production. However, the conversion of lignocellulosic biomass is challenged by its recalcitrant structure. For efficient conversion to bioethanol, it is important to study the composition of the raw lingocellulosic residues and devise appropriate delignification and saccharification strategies. The discovery of lignin degrading enzymes (lignin peroxidases, manganese peroxidases, versatile peroxidases and laccases) in cultures of the white rot fungi marked the beginning of the development of enzymatic systems for applied biomass delignification. Degradation of resulting cellulosic biomass is performed by a mixture of hydrolytic enzymes collectively known as glycosyl hydrolases (cellulases and hemicellulases), which act in a synergistic manner in biomass-degrading microorganisms. In comparison with conventional physico-chemical processes, enzymatic delignification and saccharification treatments of lignocellulosic materials are advantageous due to their specificities, low energy requirement, mild operational conditions, absence of substrate loss due to chemical modifications, and no byproduct formation. This review examines what is currently known regarding recent enzymatic technologies for delignification and saccharification of lignocellulosic materials that are used in production of second generation bioethanol.

Key words: Lignocellulosic Biomass, Bioconversion, Ligninases, Cellulases, hemicellulases, Bioethanol.

Introduction

Lignocellulosics are abundant and renewable organic materials. They consist of three major components: cellulose, hemicellulose and lignin [23]. Cellulose, the major constituent of all plant material, is a linear biopolymer of anhydroglucopyranose-molecules, linked by β-1,4-glycosidic bonds. Hemicelluloses, the second most abundant component of lignocellulosic biomass, are heterogeneous polymers of pentoses (including xylose and arabinose), hexoses (mainly mannose, less glucose and galactose) and sugar acids. Composition of hemicelluloses is very variable in nature and depends on the plant source. Lignin, the third main heterogeneous polymer in lignocellulosic residues, generally contains three aromatic alcohols including coniferyl alcohol, sinapyl and p-coumaryl [15,23,24,36].

Lignocellulosic wastes are generated in huge amounts by many industries including forestry, pulp and paper, agriculture, and food as well as from animal and municipal solid wastes. These potentially valuable lignocellulosic materials were treated as waste in many countries in the past,

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and still are today in some developing counties, which raises many environmental concerns [15]. Significant efforts, many of which have been successful, have been made to convert these residues to value added products such as bioethanol [23]. Cellulosic material in the plant cell wall is not readily available to enzymatic hydrolysis due to low accessibility of microcrystalline cellulose fibers, which prevents cellulases from working efficiently, and the presence of lignin (mainly) and hemicellulose on the surface of cellulose, which prevents cellulases from accessing the substrate efficiently [81]. Thus, enzymatic pretreatment of lignocellulosic biomass before hydrolysis is a prerequisite and this can be performed by different methods [2,5,11].

Physico-chemical methods have been used initially for cellulose degradation and they are still involved in pretreatment of lignocellulosic residues at industrial scales. However, these chemical methods are expensive, slow and inefficient. In addition, the yield of the fermentation process will be decreased because these pretreatment releases inhibitors such as weak acids, furan and phenolic compounds [11,23]. Some of these problems could be overcome by applying enzymatic delignification and saccharification methods. Indeed, many microorganisms are capable of degrading and utilizing cellulose and hemicelluloses as carbon and energy sources by secreting cellulases and hemicellulases. Moreover, an interesting group of filamentous fungi has evolved with the unique ability to degrade lignin, the most recalcitrant component of plant cell walls. These are known as white-rot fungi, which efficiently convert lignin to CO_2 by secreting ligninases [10,11,73].

This review focuses on current and latest developments in second generation bioethanol production with special emphasis on the choice of lignocellulosic substrates and the enzymatic delignification and saccharification methods that have been used for optimal, ecological and economic production of bioethanol.

Lignocellulosic biomass

Structure of lignocellulosic materials

Lignocellulosic biomass constitutes the world's largest bioethanol renewable resource. Lignocellulosics can generally be divided into three main components: cellulose (30-50 %), hemicellulose (15-35 %) and lignin (10-20 %) [51].

Cellulose is a structural linear component of a plant's cell wall consisting of long β- (1→4) linked D-glucose units that can reach several thousand glucose units in length. The presence of extensive hydrogen linkages lead to a crystalline and strong matrix structure. This cross-linkage of numerous hydroxyl groups forms the microfibrils which give the molecule more strength and compactness. Cotton, flax and pulp represent the purest cellulose sources (80-95 % and 60-80 %, respectively) while soft and hardwoods contain approximately 45 % cellulose [25,51].

Hemicellulose is an amorphous and variable structure formed of heteropolymers including hexoses (D-glucose, D-galactose and D-mannose) as well as pentose (D-xylose and L-arabinose) and may contain sugar acids namely, D-glucuronic, D-galacturonic and methylgalacturonic acids. Its backbone chain is primarily composed of xylan β (1/4)-linkages that include D-xylose (nearly 90 %) and L-arabinose (approximately 10 %). Branch frequencies depend on the nature and the source of lignocellulosic feedstocks. The dominant sugars in hemicelluloses are xylose in hardwoods and agriculture residues and mannose in softwoods. Because of the diversity of its sugars, hemicellulose requires multiple enzymes to be completely hydrolyzed into free monomers [27,49,70]. Lignin is an aromatic and rigid biopolymer with a molecular weight of 10,000 Da bonded via covalent bonds to xylans (hemicelluloses portion) conferring rigidity and high level of compactness to the plant cell wall. Lignin is composed of three alcohol monomers namely, coumaryl, coniferyl and sinapyl alcohol. Lignin is the most recalcitrant component of lignocellulosic material to degrade. It acts as a barrier by linking to both hemicelluloses and cellulose and prevents penetration of enzymes to the interior lignocellulosic structure [15,31].

Types of lignocellulosic materials used for ethanol production

There are several groups of raw lignocellulosic materials that are differentiated by their origin,

composition and structure. Lignocellulosic wastes are produced in large amounts by forestry, pulp and paper, agriculture and food industries in addition to different municipal and animal solid wastes [51]. Those derived from agricultural activities include materials such as straw, stem, stalk, leaves, lint, seed/stones, husk, shell, peel, pulp from fruits, legumes or cereals, bagasses, spent coffee grounds, brewer's spent grains, and many others. These potentially valuable materials were treated as wastes in many countries in the past, and still are today in many developing countries, which raises many environmental concerns [15]. Significant efforts have been made to convert these lignocellulosic residues to valuable products such as biofuels. Potential lignocellulosic feedstocks and their composition are summarized in Table 1.

General overview of lignocellulosic biomass convertion to bioethanol

There are four steps in the production of lignocellulosic-based ethanol: pretreatment, saccharification, fermentation and distillation. During the past two decades, there have been substantial advances in genetic and enzymatic technologies that have helped to improve these ethanol production stages [49]. Schematic picture for the conversion of lignocellulosic biomass to ethanol, including the major steps can been seen in Figure 1.

Pretreatment

Pretreatment of the lignocellulosic biomass is necessary because hydrolysis of non-pretreated materials is slow, and results in low product yield. Pretreatment makes cellulose more accessible to the cellulolytic enzymes, which in return reduces

Table 1. Composition of lignocellulosic substrates used for bioethanol production [2]

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Softwoods			
Douglas fir	50.0	2.4-3.4	28.3
Pine	44.6	5.3-8.8	27.7
Spruce	45	6.6	27.9
Hardwoods			
Black locust	41.6	17.7	26.7
Hybrid poplar	44.7	18.6	26.4
Eucalyptus	49.5	13.1	27.7
Populus tristis	40-49.9	13-17.4	18.1-20
Crop Residues			
Corn cobs	45.0	35.0	15.0
Corn fiber	14.3	16.8-35.0	8.4
Corn stover	36.8-39	14.8-25.0	15.1-23.1
Cotton gin trash	20.0	4.6	17.6
Grasses (sugarcane, sorghum bagasse)	25-50	25-50	10-30
Rice straw	35-41	25-14.8	9.9-12.0
Rice hults	36.1	14.0	19.4
Wheat straw	30-38	21-50	20-23.4
Herbaceous Materials			
Bermuda grass	25.0	35.7	6.4
Switchgrass	31-32	20.4-25.2	14.5-18.1
Cellulose Wastes			
Newsprint	40-64.4	4.6-40	18.3-21
Paper	85-99	0.0	0-15

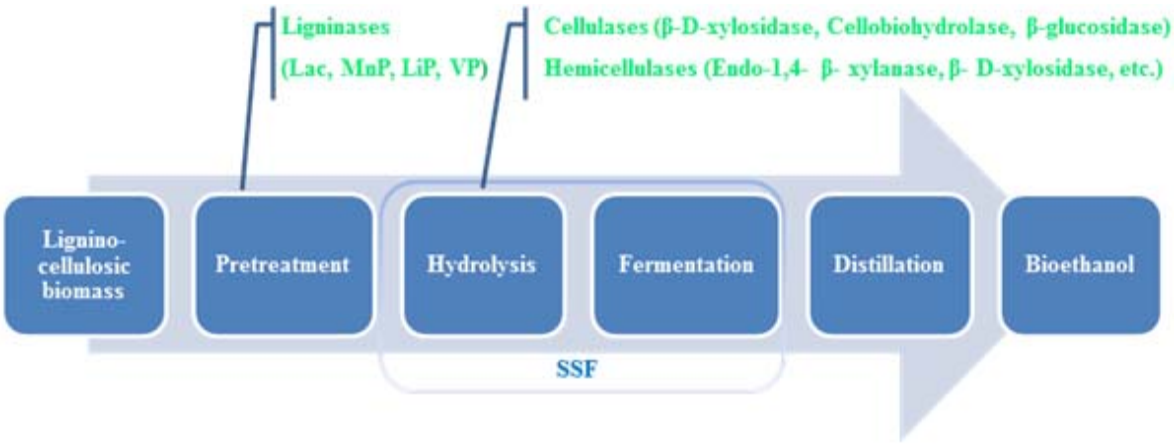

Figure 1. Schematic flowsheet for the conversion of ligninocellulosic biomass to bioethanol. Application of green technologies using ligninases, cellulases and hemicellulases for delignification and saccharification steps were presented in green

hydrolytic enzyme requirements and, thus, the cost of ethanol production. Numerous pretreatment methods have been reported and are generally categorized as mechanical, physicochemical, chemical, and biological processes or combinations of these methods [15,23]. Most of these pretreatments can liberate hemicellulose and cellulose from the cell wall but some of them remain economically unfeasible due to key technical issues. Also, they are not all able to overcome the recalcitrant lignin structure found mainly in wood-based feedstocks [51]. Biological pretreatment uses microorganisms and their ligninolytic enzymes have the advantages of a low-energy demand, minimal waste production and a lack of environmental effects [23].

Hydrolysis of pretreated biomass

The released cellulose and hemicelluloses after application of a suitable pretreatment are hydrolyzed into free monomer sugars (hexoses and pentoses) readily available for ethanolic fermentation [39]. There are two types of saccharification processes that involve either acidic or enzymatic reactions [71]. The acidic hydrolysis treatment can be divided into dilute or concentrated acid hydrolysis. Dilute hydrolysis (1-3 %) requires a high temperature of 200-240°C to disrupt cellulose crystals. It is followed by hexose and pentose degradation and formation of high concentrations of toxic compounds including furfural and phenolics detrimental to an effective saccharification. Concentrated acid treatment, the more prevalent hydrolysis method, has been considered to be the most practical approach. Unlike dilute acid treatment, concentrated acid hydrolysis is not followed by high concentrations of inhibitors and produces high yields of free sugars up to 90 %. However, this method requires large quantities of acid as well as costly acid recycling, which makes it commercially less attractive [47]. Enzymatic hydrolysis has been considered key to cost-effective cellulosic bioethanol in the long run, and the reaction is carried out with mainly cellulase and hemicellulase enzymes. The advantage of using biocatalysts over acids is to eliminate corrosion problems and to decrease maintenance costs with mild processing conditions to give high yields. However, improving hydrolytic activities and finding stable extremozymes capable of tolerating extreme conditions has become a priority in many recent studies [5,8,13,14,21,37,50,52,58,63,81].

Fermentation

The saccharification end-products including monomeric hexoses (glucose, mannose and galactose) and pentoses (xylose and arabinose) will be fermented to valuable ethanol. Among these products, glucose is the most abundant, followed by xylose or mannose and other lower concentration sugars [15]. The hydrolysis and fermentation steps can be performed separately or simultaneously. The advantage of the separate hydrolysis and fermentation method is that both processes

can be optimized individually (e.g. optimal temperature is 45-50°C for saccharification, whereas it is 30°C for ethanolic fermentation). However, its main drawback is the accumulation of enzyme-inhibiting end-products during the hydrolysis process. The simultaneous hydrolysis and fermentation method combines the enzymatic saccharification of cellulose to glucose and its eventual fermentation by yeast to ethanol in the same vessel. Rapid conversion of the glucose into bioethanol by yeast results in faster rates and higher yields compared to the separate hydrolysis and fermentation method. However, the main drawback of the simultaneous hydrolysis and fermentation method is the need to compromise processing conditions particularly temperature and pH. The development of recombinant yeast strains with improved thermotolerance can enhance the performance of this method [15,23]. More progress has been made in recent years in modifying various microbes including yeast (e.g., *Saccharomyces cerevisiae*, *Scheffersomyces (Pichia) stipites)* and bacteria (e.g., *Zymomonas mobilis, Klebsiella oxytoca*) to make them capable of fermenting both hexoses (C6; glucose, galactose, and mannose) and pentoses (C5 sugars; xylose and arabinose) at comparatively high yields [48].

Ethanol recovery

The fermentation product from the glucose and pentose fermentation is called ethanol broth. In this step the ethanol is separated from the other components in the broth through a distillation process. Water can be separated via a condensation procedure and ethanol distillate recaptured at a concentration of 95%. A final dehydration step removes any remaining water from the ethanol [51].

Oxidative enzymes for lignin removal

Lignin is composed of non-phenolic and phenolic structures. White-rot basidiomycetes such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Trametes Trogii*, *Pleurotus ostreatus*, *Lentinula edodes*, *Irpex lacteus*, *Fomes fomentarius* and *Cerrena maxima* have been found to be the most efficient lignin-degrading microorganisms studied. It has been reported that lignin degrading fungi secreted enzymes collectively termed "ligninases" (Figure 2). These include two ligninolytic families; phenol oxidases (laccases) and peroxidases [lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP)]. In delignification process, ligninolytic enzymes have been applied in different methods as

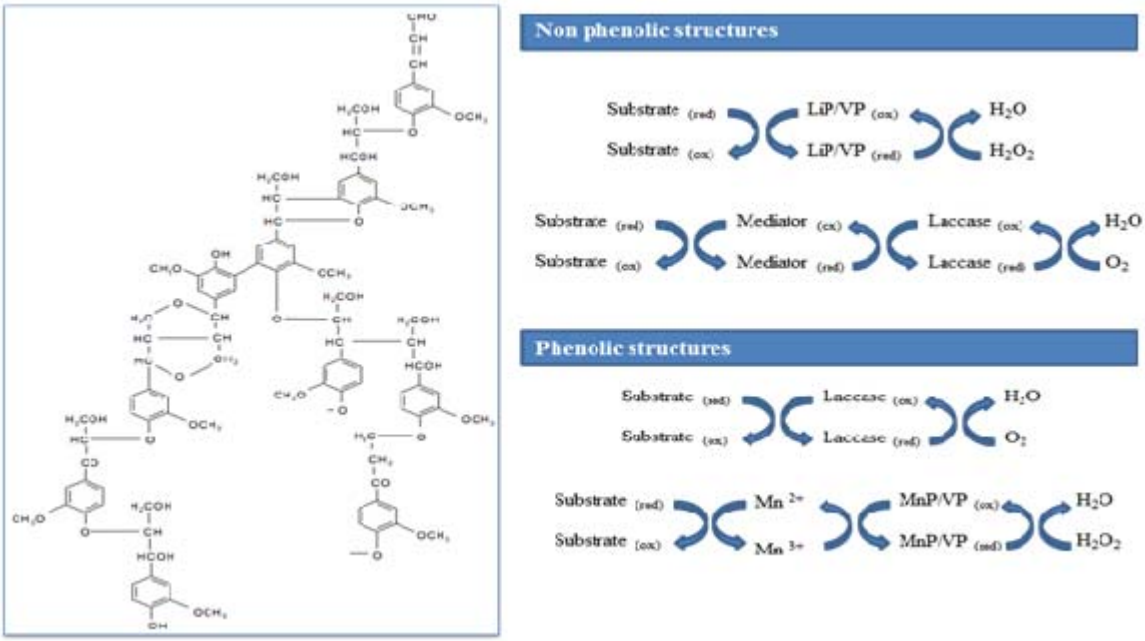

Figure 2. Schematic representation of the ligninolytic system (for explanation see text). Updated from Abdel-Hamid *et al.*[1]

fungal delignification, enzymatic delignification, laccase-mediator system (LMS), and integrated fungal fermentation (IFF) [2,7,47,54,56,65,67].

Lignolytic peroxydases

LiP (EC 1.11.1.14) catalyzes the H_2O_2-dependent oxidative depolymeriza-tion of lignin. It was first discovered in the the white-rot fungus, *P. chrysosporium*. Multiple LiP isoforms exist in *P. chrysosporium* and other white-rot fungi such as *Phanerochaete sordida*, *T. versicolor*, *Phlebia radiata*, and *Phlebia tremellosa* [1]. LiPs are stronger oxidants because the iron in the porphyrin ring in theses enzymes is more electron deficient than in classical peroxidases. Therefore, LiPs can oxidize aromatic substrates which are moderately activated, whereas classical peroxidases act only on strongly activated aromatic sub-strates. LiPs catalyse the oxidation of the major non-phenolic structures of lignin, which constitute up to 90% of the lignin polymer [1,32,57].

MnP (Mn(II):hydrogen-peroxide oxidoreductase, EC 1.11.1.13) is another key oxidative enzyme for lignin degradation [33]. MnP oxidizes Mn^{2+} to Mn^{3+} in a reaction that is dependent on H_2O_2; the manganese ion is chelated to an organic acid. Mn^{3+} is a strong oxidant (1.54 V) and can act on a variety of phenolic compounds as a redox mediator through the lignin matrix. The MnP system can also oxidize nonphenolics, including the model compounds veratryl alcohol and benzyl alcohol. The redox potential of chelated Mn^{3+} depends on the chelator. Manganese ion chelators include organic acids such as malonic and oxalic acids. It has also been reported that MnP can cleave non-phenolic lignin compounds via the action of small mediators such as thiyl or lipid radicals. In contrast to LiP, MnP is not capable of oxidizing the high recalcitrant non-phenolic structures [1,33,34,55,68].

VP (EC 1.11.1.16) is a heme-containing ligninolytic peroxidase with hybrid molecular architecture combining different oxidation-active sites [64]. VP was first reported and characterized in the white-rot fungus *Pleurotus eryngii* [56]. VP has only been described in species of the genera *Pleurotus* and *Bjerkandera* [59]. VP enzymes have the catalytic activities of both MnP and LiP and are able to oxidize Mn^{2+} like MnP, and high-redox potential non-phenolic compounds like LiP. The catalytic versatility of VP permits its application in Mn^{3+}-mediated or Mn-independent reactions on both low and high-redox potential aromatic substrates. Although VP from *P. eryngii* catalyzes the oxidation of Mn^{2+} to Mn^{3+} by H_2O_2, it differs from classical MnPs by its manganese-independent activity, enabling it to oxidize substituted phenols, as well as the LiP substrate, VA [1,64,69].

Laccases and the mediator system

Laccase or benzenediol:oxygen oxidoreductase (EC 1.10.3.2) is a blue copper enzyme with broad substrate specificity that oxidizes phenols, anilines, and aromatic thiols, with the concomitant four electron reduction of O_2 to H_2O [22]. The enzyme occurs in fungi, plants, trees, insects, and bacteria. Laccases have relatively low redox potentials ($\leq$0.8 V) compared to ligninolytic peroxidases (>1 V) [18]. Laccase was long considered to degrade only phenolic lignin and phenolic lignin model compounds, since nonphenolic compounds with redox potentials greater than 1.3 V could not be oxidized by laccase directly [20]. Given that phenolic lignin structures account for 10-20 % of the lignin in the plant cell wall, laccase was therefore not expected to play a role as a delignifying agent [75]. However, the combination of laccase and syringaldehyde was shown to oxidize veratryl alcohol, a nonphenolic lignin model compound [42]. This result was confirmed by Bourbonnais and Paice [16] who reported that laccase could degrade nonphenolic lignin model compounds when used in conjunction with the redox mediator 2,2-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS). Other mediators including, 1-hydroxybenzotriazole (HBT), violuric acid (VIO), and N-hydroxyacetanilide (NHA) are being particularly effective in lignin degradation [18]. Given the high cost of HBT and ABTS, the synthetic mediator NHA has been described as both cost effective and biodegradable, with another particular advantage that enzyme can retain about 80 % of its initial activity in an laccase mediator system stage with this mediator. Naturally occurring phenolic mediators such as acetosyrignone and syringalde-hyde can provide up to 25 % lignin

removal [19]. Overall, the ideal mediator would be a small molecule that is oxidizable to a stable radical that would not inactivate the enzyme. In addition, laccase mediators should be environmentally benign, available at low cost, and would be needed in catalytic rather than stoichiometric quantities due to recycling without degeneration [12,18,22].

Integral fungal fermentation

Laccases are the most enzymes used in delignification pretreatments followed by MnPs and LiPs; nevertheless, mixtures of two or three ligninolytic enzymes have been also used. In this case, the synergetic relationship between the ligninolytic enzymes improved biomass delignification [65]. Integrated fungal fermentation (IFF) is a consolidated process where a fungus or a group of fungi convert biomass into ethanol without the participation of other microorganisms or treatments [38]. For an example, the white rot fungus, *Phlebia* sp. MG-60 has the ability to selectively transform lignin under solid state fermentation and produce ethanol from delignified biomasses under submerged fermentation [39]. This fungus has been evaluated in the production of cellulosic ethanol from many feedstocks such as hardwood kraft pulp, waste newspaper, sugarcane bagasse, and hard wood [38,39,43]. *Phlebia* sp. MG-60 produced an ethanol yield between 30 % and 70 % depending of the type of biomass utilized [44]. On the other hand, fungi co-culture is an alternative to generated and integrated process for ethanol production. In this case, ethanol is produced by mixing two types of fungi to performing delignification, hydrolysis, and fermentation. The two white rot fungi *Coprinus comatus* and *Trametes reesei* were cultivated to produce ethanol from corn stover. In this case, coculture achieved greater delignification and cellulose conversion than monoculture [53]. Table 2 resumes the application of these ligninolytic enzymes for delignification process.

Enzymatic saccharification by glycosyl hydrolases: cellulases and hemicellulases

Cellulases

Enzymatic saccharification using cellulases is a key step in the bioconversion of lignocellulosic biomass to ethanol, since it is the most time and energy consuming [61,62]. Although hemicellulases and accessory enzymes are also important parts and should be present in multienzymatic system for hydrolysis of lignocellulosic residues, cellulases are the major components of this hydrolytic system because of the recalcitrant crystalline nature of cellulosic fibers demanding high cellulase loadings [29]. Many fungal species have the ability to degrade cellulose by producing extracellular cellulose-degrading enzymes including endo-cleaving (endoglucanases) and exo-cleaving (cellobiohydrolases) [26]. Endoglucanases break down cellulose chains internally in a random manner whereas cellobio-hydrolases act preferentially on chain ends. The reaction products are mostly a disaccharide known as cellobiose and, to a lesser extent, cello-oligosaccharides, which will be further hydrolyzed by the third group of enzymes called β-glucosidases (figure 3). Fungal cellulases are the most economic and available sources to meet the industrial scale, because fungi can grow on an inexpensive solid state media such as agriculture and food industries by-products. Recent developments in enzyme engineering have revolutionized the development of commercially available cellulases into better industrial catalysts [76]. Therefore currently, most industrial cellulases are produced by engineered fungal strains, specifically *Trichoderma reesei* and *Humicola insolens,* due to their high yields and specific activities [13]. Generally, the fungus *T. reesei* secreted at least two cellobiohydrolases (CBHI and CBHII), five to six endoglucanases (EGI to EGVI), beta-glucosidase (BGL I and II), two xylanases and various accessory hemicellulases [79,81].

Hemicellulases

Hemicellulose consists of a combination of complex branched heteropolymers. Glucomannan and xylan constitute the largest components in softwood and hardwood, respectively. Other components include xyloglucan, glucomannan, galactoglucomannan and arabinogalactan [13]. Hemicelluloses are hydrolysed by a combination of enzymes releasing valuable fermentable sugars such as pentoses (D-xylose and D-arabinose)

Table 2. Delignification pretreatment using ligninolytic enzymes

Lignocellulosic biomass	Microorganism	Lignolytic enzyme	Delignification (%)	Reducing sugar yield (%)	Ethanol yield (%)	Reference
Wheat straw	*Ceriporiopsis subvermispora* *Irpex lacteus*	MnP, LiP Laccase	34 % 39 %	69 % 66 %	90 %	Salvachúa *et al.*, [72]
Sugarcane bagasse	*Pleurotus ostreatus IBL-02*	MnP, LiP, Laccase	33.6 %	72.9 %	16.3 g/L	Asgher *et al.*, [9]
Corn stover	*Irpex lacteus*	MnP	25.48 %	308.98 mg/g	144.03 mg/g	Song *et al.*, [77]
Hardwood kraft pulp	*Phlebia sp. MG-60*	Integrated fungal fermentation	-	-	71.8 %	Kamei *et al.*, [38]
Waste newspaper	*Phlebia sp. MG-60*	Integrated fungal fermentation	-	-	51.1 %	Kamei *et al.*, [38]
Oak wood	*Phlebia sp. MG-60*	Integrated fungal fermentation	-	-	43.9 %	Kamei *et al.*, [39]
Cotton gin trash	*Trametes versicolor*	LMS	27 %	41 %	64 %	Plácido and Capareda, [66]
Ricinus communis	-	Laccase	85.69 %	288.83 mg/g	-	Mukhopadhyay *et al.*, [60]
Eucalyptus globulus	*Pycnoporus cinnabarinus*	LMS	27 %	-	-	Ibarra *et al.*, [36]
Elephant grass	*Trametes villosa*	LMS	36 %	70.7 %	16.2 %	Gutiérrez *et al.*, [30]
Eucalypt wood	*Trametes villosa*	LMS	58 %	57.8 %	12.3 %	Gutiérrez *et al.*, [30]
Corn stover	*Ceriporiopsis subvermispora*	Laccase	>25 %	56 %	-	Wan and Li, [80]
Switchgrass	*Ceriporiopsis* subvermispora	Laccase	>25 %	37 %	-	Wan and Li, [80]

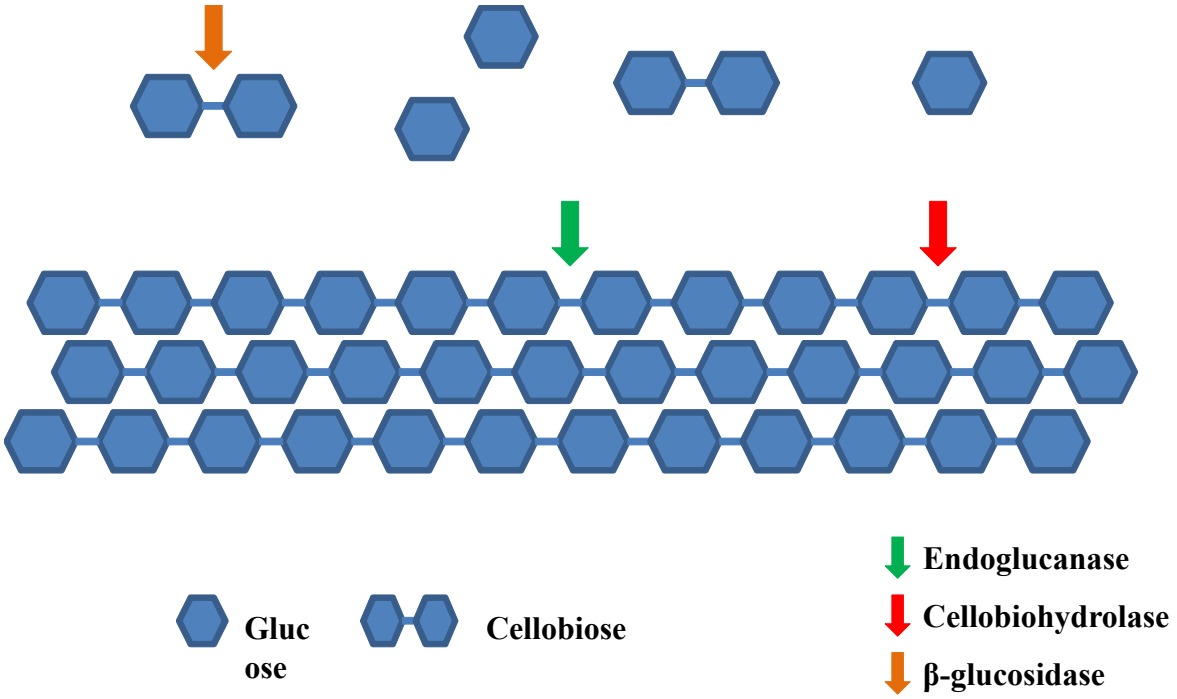

Figure 3. Schematic representation of the cellulolytic system.
The arrows represent each enzyme active for a determined substrate [26]

and hexoses (D-glucose, D-galactose and D-mannose) as well as sugar acids. In hardwood degradation, β-xylanases, β-xylosidases and α-glucuronidases are involved in degradation of the main backbone. However, accessory enzymes such as α-arabinofuranosidases, α-galactosidases, feruloylesterases and acetylxylan esterases could be key enzymes removing hemicellulosic fraction and increasing accessibility of cellulases to the cellulose fibres and improving the enzyme saccharification process (figure 4). For softwood the main hemicellulases are mannanases and β-mannosidases, while the auxiliary enzymes are likely α-galactosidases and acetylxylan esterases. These enzymatic compositional differences condition the enzyme formulation for the efficient degradation of various biomass stocks [46]. Several species of bacteria such as *Agrobacterium*, *Bacillus*, *Bifidobacterium*, *Clostridium*, *Streptomyces*, *Cellulomonas*, *Geobacillus* species, and fungi such as *Aspergillus* and *Trichoderma* species are able to produce hemicellulases [74]. The main hemicellulase-producing microorganisms used for bioethanol production are *Aspergillus niger*, *Trichoderma reesei* and *Trichoderma viride* [17]. For extensive reviews on xylanases and cellulases and their application in energy production, the reader is referred to [4,13,29,48,81].

Conclusion and future prospect

Production of second-generation bioethanol as alternative fuel using lignocellulosic-biomass materials is one of the most desirable goals to overcome the fossil fuel crisis. The bioconversion of lignocellulose into ethanol is achieved in three steps: biomass delignification, carbohydrates depolymerization and fermentation to ethanol using the liberated sugars. Biological treatment using lignino-hemi-cellulolytic microorganisms has been proposed, to replace the physico-chemical treatments. Enzymatic treatments can also be used for the removal of inhibitors prior to the fermentation process.

The advantages of using microbial and enzymatic treatments include milder reaction conditions, higher production yield, fewer side reactions, and less energy consumption. However, engineering of oxidative and hydrolytic enzymes for improved catalytic efficiency will be needed to make such systems more competi-

tive. The development of extremophilic biocatalysts is also one of the most recent contributions in this energy field. Developments in genetic systems and strain production will continue to push new innovations that enable developers to achieve a sustainable supply of cellulosic bioethanol.

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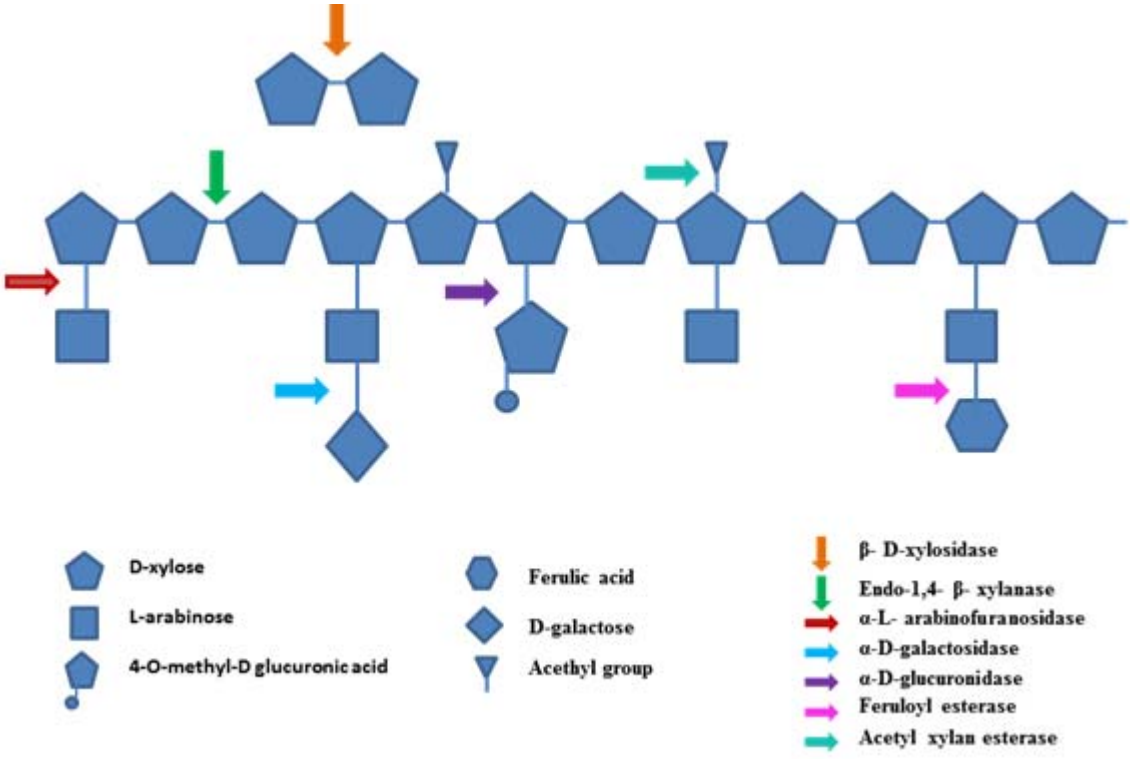

Figure 4. Schematic representation of the hemicellulolytic system. The arrows represent each enzyme active for a determined substrate [26]

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