Engineering in Life Sciences

Eng. Life Sci. **2016**, *16*, 1–16 www.els-journal.com

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Review

Lignocellulosic biomass: Biosynthesis, degradation, and industrial utilization

Lignocellulose biomass derived from plant cell walls is a rich source of biopolymers, chemicals, and sugars, besides being a sustainable alternative to petrochemicals. A natural armor protecting living protoplasts, the cell wall is currently the target of intense study because of its crucial importance in plant development, morphogenesis, and resistance to (a)biotic stresses. Beyond the intrinsic relevance related to the overall plant physiology, plant cell walls constitute an exquisite example of a natural composite material that is a constant source of inspiration for biotechnology, biofuel, and biomaterial industries. The aim of the present review is to provide the reader with an overview of the current knowledge concerning lignocellulosic biomass synthesis and degradation, by focusing on its three principal constituents, i.e. cellulose, hemicellulose (in particular xylan), and lignin. Furthermore, the current industrial exploitation of lignocellulose from fast growing fibre crops (such as hemp) is highlighted. We conclude this review by suggesting approaches for further research to fill gaps in our current knowledge and to highlight the potential of biotechnology and bioengineering in improving both biomass biosynthesis and degradation.

Keywords: Biocomposites / Biofuel / Biotechnology / Enzyme engineering / Plant cell wall

Additional supporting information may be found in the online version of this article at the publisher's web-site

Received: October 5, 2014; *revised:* June 9, 2015; *accepted:* July 23, 2015

DOI: 10.1002/elsc.201400196

1 Plant cell walls: Through thick and thin

The plant cell wall is a structure characterized by a mesh of polysaccharides, structural proteins, and phenolic compounds that besides protecting the plant cell against external stresses, provides structural and mechanical support to plant tissues. The chemical composition and mechanical properties make plant cell walls not only a rich source of chemicals and fermentable sugars

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Abbreviations: CBM, carbohydrate binding module; ***CesA***, cellulose synthase gene; **CESA**, cellulose synthase protein; **GT**, glycosyltransferase; **PCW**, primary cell wall; **SA**, salicylic acid; **SCW**, secondary cell wall; **TC**, terminal complex

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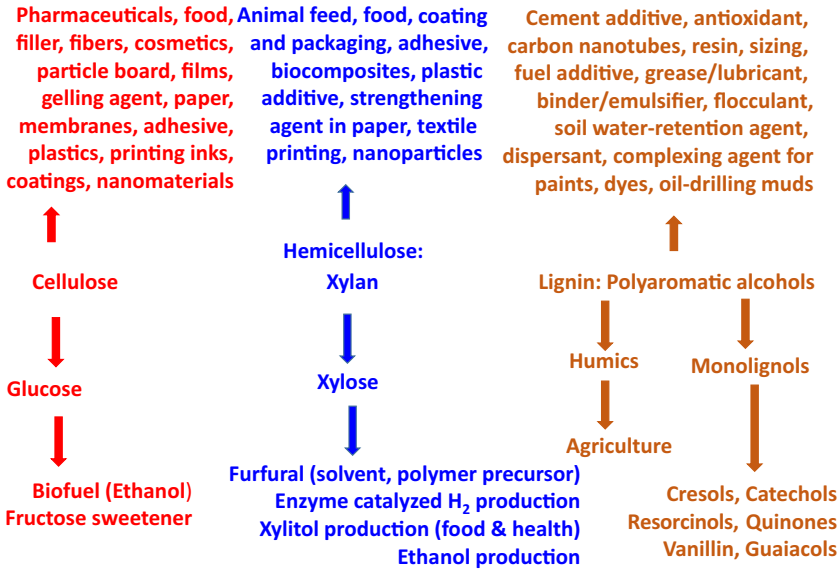

Figure 1. Applications of plant cell wall components and their degradation products. Red, cellulose; blue, xylan; and brown, lignin.

for the production of biofuels, but also biopolymers for various industrial applications (Fig. 1).

Plant cell walls are classified into two types, i.e. primary and secondary cell walls (PCW and SCW), which differ both in their chemical composition and in the physiological roles they play. An additional type of cell wall (more precisely a cell wall layer), called "*gelatinous*" (or G-layer) found in tension wood that is particularly rich in crystalline cellulose and poor in lignin [1] can be observed in response to mechanical stimuli, e.g. tensional stress due to bending. This type of wall, interestingly, occurs naturally in the bast fibres of herbaceous crops like hemp (*Cannabis sativa* L.) and flax (*Linum usitatissimum* L.), where they provide mechanical support to the phloem tissue and envelop a woody core (for a recent review see [2]). These crops have gained a lot of interest in recent years because they represent a rapidly available source of lignocellulosic biomass where the presence of gelatinous fibres with low lignin content provides less hindrance to enzymatic hydrolysis [1].

PCWs are laid down around dividing and elongating cells and consist largely of polysaccharides (cellulose, hemicelluloses, and pectin) and approximately 10% protein in addition to phenolic esters linked to wall polysaccharides [3]. The structure of PCWs is thin and flexible, thus making it ideal to encase growing and expanding plant cells. Specific cells, such as the conductive xylem vessels and sclerenchyma fibres, synthesize SCWs that are made up of a cross-linked matrix of cellulose, hemicelluloses, and lignin, and are laid down on the interior of the PCWs (Fig. 2A). Lignin forms the fundamental scaffold of fibres and vessels found in the xylem. Its occurrence in PCWs and middle lamella has also been documented (e.g. [4,5]). In addition to providing mechanical support, SCWs fulfil critical biological processes, such as water and nutrient transport, anther dehiscence, silique shattering, plant organ movement, and defense against pathogens [6].

Wood, one of the most important human commodities and the major C sink of terrestrial ecosystems, is mainly composed of secondary walls (50% cellulose, 25% lignin, and 25%

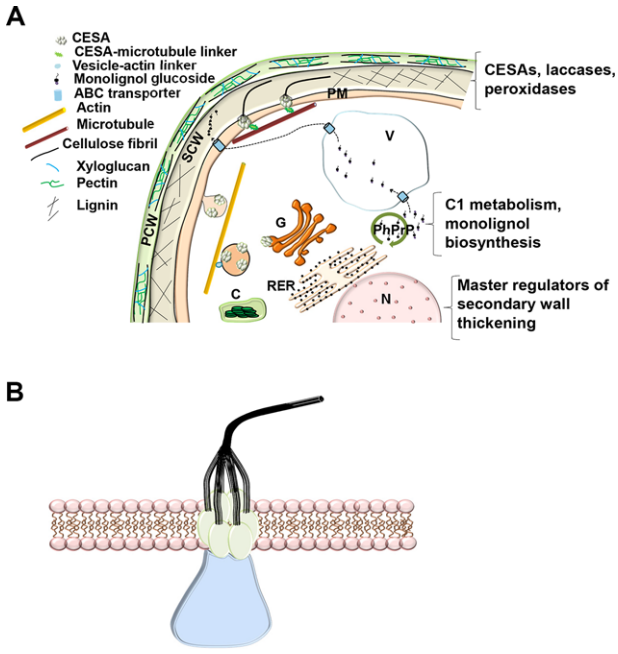

Figure 2. Schematic representation of the principal steps involved in SCW formation [133]. (A) The CESA complex delivery to the plasma membrane (PM) and its interaction with the cytoskeleton is shown. The biosynthesis of the monolignols through the phenylpropanoid pathway (PhPrP) and their transport via ABC transporters is also indicated. N, nucleus; RER, rough endoplasmic reticulum; G, Golgi complex; V, vacuole; C, chloroplast; PCW and SCW indicate primary and secondary cell wall respectively. (B) Cartoon depicting the rosette TC (light green) embedded in the lipid bilayer (pink). The cytoplasmic domain is shown in light blue (not to scale). The six particles of the complex are drawn in the process of synthesizing 36 glucan chains (the particle subunits are not represented) that assemble into a microfibril (black).

hemicelluloses, [7]). Understanding SCW deposition is important from the biotechnological point of view because of the potential of second-generation biofuel feedstock (such as short-rotation hardwoods, e.g. *Populus*, *Eucalyptus*) and the widespread use of lignocellulosic biomass in pulp, paper, and cellulose-derived products.

2 Cellulose biosynthesis and degradation

Cellulose is a linear polysaccharide of glucose joined by β-1,4-glycosidic linkages. The absence of side chains enables the long glucose chains to pack tightly together due to van der Waals interactions and hydrogen bonds via the -OH groups, thereby creating crystalline nonsoluble microfibrils [8]. In addition to containing crystalline (ordered) regions, cellulose also includes amorphous (disordered) regions. It is generally assumed that a cellulose microfibril is composed of 36 chains [9] and this model is based on the six-fold symmetry of the rosette terminal complex (TC; Fig. 2B). The rosette TC is a membrane-localized nanomachine found at the tip of growing cellulose microfibril that imprints on the freeze-fractured plasma membrane and is responsible for the biosynthesis of cellulose in higher plants [10]. The rosette is believed to be pre-assembled in the Golgi apparatus [11, 12] and then transported and inserted into the plasma membrane with the intervention of microtubules [13] (Fig. 2A). Rosettes have been shown to move in the plasma membrane along tracks that are aligned with the underlying cortical microtubular network [14] (Fig. 2A).

The rosette TC is composed of six particles (Fig. 2B), each of which may contain up to six catalytic subunits, encoded by the cellulose synthase genes. It is characterized by a portion of about 24 nm embedded in the membrane [10] and by a domain of approximately 45–50 nm of diameter that faces the cytoplasm [15, 16] (schematized in Fig. 2B).

CesA genes encode for processive Family 2-glycosy ltransferases (GT2s) (http://www.cazy.org/GT2.html) that typically possess eight transmembrane domains and show the presence of the conserved D, DxD, D, QxxRW motif involved in activity. Plant cellulose synthase proteins (CESAs) display specific sequences that are not present in bacterial proteins, such as the plant conserved sequence (P-CR) and the class-specific region. Recently, a tertiary model of a cotton CESA revealed that the P-CR and class-specific region (CSR) are localized at the interface of the monomers and are implicated in oligomerization [17; Fig. 3A]. The cytoplasmic region of plant CESAs additionally contains a Zn-finger domain involved in forming interactions with other CESAs. Very recently a soluble 57 kD recombinant catalytic domain of rice CESA8 was analyzed, which showed that the dimer is the fundamental scaffolding unit of the cellulose synthase complex, where each monomer synthesizes a single glucan chain [18].

In SCWs, the presence of CESA4, CESA7, and CESA8 is required [19], whereas in PCW the interaction of CESA1, CESA3, and CESA6 (or CESA1, CESA3, and CESA6-releated proteins) is necessary [20]. However, a recent study has shown that complexes with mixed primary and secondary CESAs are also functional, suggesting that primary and secondary CESAs might be part of the same complex at specific stages of the development [21].

Beside the interactions established by different CESA subunits within a rosette complex, a cohort of other proteins is required to regulate and coordinate the deposition of cellulose [22, 23]. The co-expression of a set of genes with specific cellulose synthase genes (*CesA*s) can be a good indicator of its functional association in cell wall biosynthesis. For instance, groups of genes often show overlapping expression patterns in specific tissues during SCW deposition, namely those linked to the cytoskeleton, signaling, transcription factors, lignin, and xylan biosynthesis [24]. The co-expression network analysis has therefore emerged as a powerful tool to unravel functionally associated groups of genes during specific stages of wall biosynthesis.

The enzymatic degradation of cellulose is a process that yields fermentable glucose and is therefore important from the biotechnological point of view [25, 26]. Degradation of cellulose is catalyzed by the extracellular cellulase enzyme system and requires the synergistic action of three components: endo-1,4-β-glucanases (EC 3.2.1.4), exo-1,4-β-glucanases or cellobiohydrolase (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) [27]. To achieve increased cellulose degradation, some bacterial and fungal glycoside hydrolases show multimodular architecture comprising a carbohydrate binding module (CBM), a linker and a catalytic domain. As an example of a modular cellulolytic enzyme, the structure and function of an exo-cellulase is depicted in Fig. 3(B). As a result of firm binding to the cellulose, the CBM helps promote its degradation by increasing its local concentration and bringing the enzyme in close and prolonged association with its recalcitrant substrate [28, 29]. Endo-1,4-β-glucanases cleave internal bonds of the glucose chains, exo-1,4-β-glucanases act on the reducing or nonreducing end (Fig. 3B), whereas β-glucosidases catalyze the last step of cellulose hydrolysis as they act on the products (mainly cellobiose) generated by the other two classes of enzymes [27]. The amorphous regions of cellulose are more easily attacked by hydrolytic enzymes, therefore the pretreatment of biomass in order to convert crystalline to amorphous cellulose, is necessary to increase the hydrolysis efficiency. Enzymes capable of attacking crystalline polysaccharides have also been identified and described. Recently a protein, CBP21 (from chitin-binding protein), acting on crystalline chitin was found to increase the catalytic conversion of the substrate by introducing chain breaks and creating a chain end with a C1-oxidized sugar [30]. This protein belongs to the AA10 family (auxiliary activity 10, formerly carbohydrate-binding module family 33, CBM33; http://www.cazy.org/AA10.html) that is rare in eukaryotes and is structurally similar to the AA9 family (formerly glycoside hydrolase family 61, GH61), which is instead well represented in eukaryotes, especially in fungi [27]. Family AA9 enzymes of fungal origins from *Phanerochaete chrysosporium* and *Neurospora crassa*, are copper-dependent lytic polysaccharide monooxygenases (LPMOs) [31–33] that are able to oxidize the C1, C4, and C6 carbon [34–36]. Interestingly, it was recently demonstrated that a member of the AA9 family, *Nc*LPMO9C from *N. crassa*, can also act on other substrates, such as hemicelluloses, revealing the additional role of oxidative enzymes during plant biomass degradation [37]. Some AA10 representatives, like CelS2 from *Streptomyces coelicolor*, are active on cellulose and work in synergy with cellulases, thus increasing the rate of

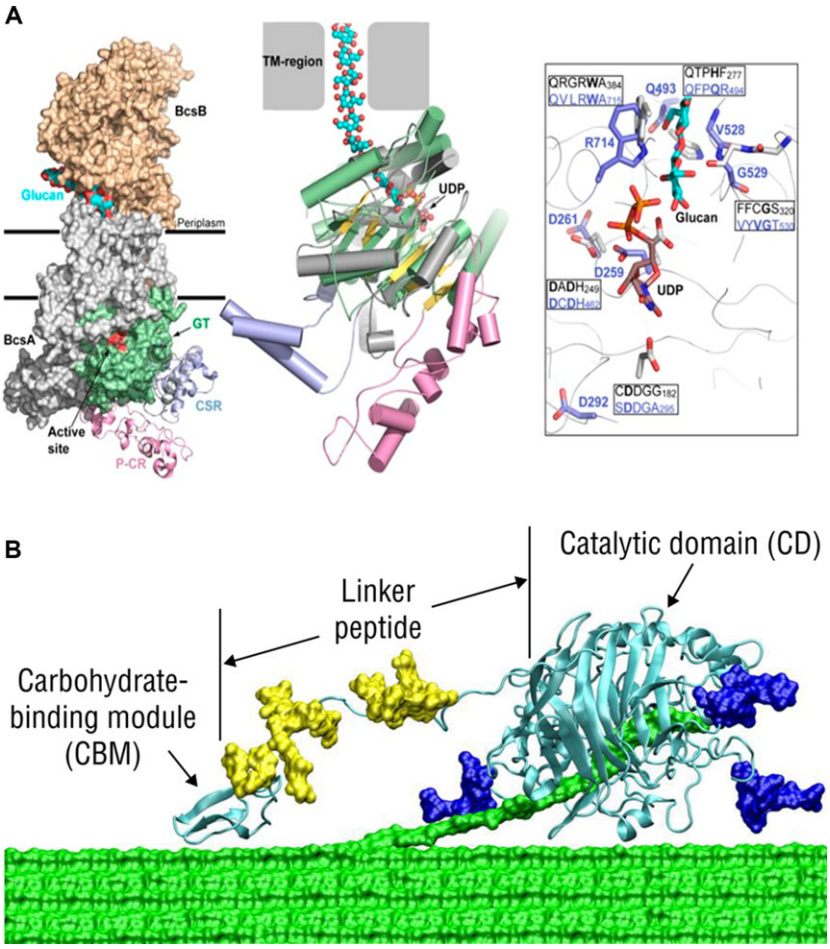

Figure 3. Models showing the synthesis and hydrolysis of cellulose. (A) Synthesis of cellulose by cellulose synthase (CESA) enzyme complex. Left: Surface representation of the *Rhodobacter sphaeroides* BcsA (PDB ID 4HG6) superimposed on the *Gossypium hirsutum* CESA cytosolic structure (glycosyl transferase, GT-domain in green). Center: Superimposition of the bacterial and the cotton GT-domains (in green and grey/yellow, respectively). The plant conserved sequence (P-CR) and the class-specific region (CSR) are colored pink and light blue whereas UDP and glucan are shown as spheres. Right: Conserved sequence motifs forming the binding site for UDP. The acceptor glucan are compared between cotton (blue letters) and *R. sphaeroides* (black letters) with residues depicted in bold and blue (Gh506), gray (BcsA), rust (UDP) and cyan (glucan). Reproduced with permission from [17] Sethaphong, L., Haigler, C. H., Kubicki, J. D., Zimmer, J. et al., Tertiary model of a plant cellulose synthase. *Proc. Natl. Acad. Sci. U.S.A.* 2013, *110*, 7512–7517. (B) Hydrolysis of cellulose by modular exo-cellulase (cellobiohydrolase, CBH) showing a carbohydrate-binding molecule (CBM, left); a linker peptide (middle) with attached polysaccharides (yellow) and a catalytic domain (CD, right) linked to polysaccharides (blue). Green, cellulose chain; blue, disaccharide cellobiose product. The CBH figure is reprinted with permission from the National Renewable Energy Laboratory, from NREL publication: http://www.nrel.gov/docs/fy12osti/53932.pdf

lignocellulosic biomass conversion by three-fold [38]. For a detailed discussion on the role of enzymes in the modification of SCW polysaccharides, readers are referred to an excellent review [39].

3 Xylan biosynthesis and biotechnology prospects

Among the hemicelluloses found in SCWs is xylan [40, 41]. Xylan is characterized by a linear backbone of β-1,4-linked xylosyl residues substituted with acetyl, glucuronic acid, 4-*O*-methylglucuronic acid, and arabinose, depending on the species and the type of wall [41]. As plant cells grow and expand, xylan undergoes remodeling by the action of xylosidases and arabinosidases. Xylan establishes interactions with cellulose, lignin, pectin, and arabinogalactan proteins. The association with the last two is known as Arabinoxylan Pectin Arabinogalactan Protein 1, APAP1 ([41] and references therein).

The biosynthesis of xylan takes place in the Golgi apparatus and involves the action of several enzymes. Type II GTs belonging to families 43 and 47 (IRX9, IRX14 and IRX10, from Irregular Xylem) synthesize the backbone [42–44], while GTs from family 8 catalyze the addition of glucuronic acid residues [41 and references therein,45]. Mutations in the genes coding for GTs involved in the backbone synthesis have been shown to cause strong phenotypic changes, such as dwarfism, a consequence linked to impaired water and nutrient transport caused by xylem vessel collapse [46].

The pattern of decorations with glucuronic acid residues is important in determining its interaction with cellulose. Two GT8 catalyzing additions of glucuronic acid residues have been described, GUX1 and GUX2 (glucuronic acid substitution of xylan). GUX1 was shown to add decorations to evenly-spaced xylosyl residues (determining the formation of the major domain of xylan), while GUX2 performs additions to even- and odd-spaced residues forming the minor domain [45]. Recent work employing molecular dynamics simulation has revealed that the pattern of acetylation is an important factor determining the docking of xylan with cellulose [47].

Acetylation of xylan is performed by reduced wall acetylation family proteins [48, 49], which are putative acetyl-CoA transporters. More recently, Eskimo/TBL29 has been proposed as a xylan acetyltransferase [50]. A protein containing a domain of unknown function (DUF579) has been shown to perform 4-*O*-methylation of glucuronic acid [51], while GT61 members

have been proposed as candidates performing arabinofuranose residue addition [52].

Xylan bioengineering is currently an important topic of study, since its presence in plant lignocellulosic biomass strongly impacts the overall efficiency of enzymatic hydrolysis [40, 41]. Traditionally, biotechnological processes of lignocellulosic biomass have made use of only the hexose component of the cellulose fraction. Pentoses derived from the hemicellulosic fraction may represent up to 40% of lignocellulosic biomass and are more difficult to ferment. The use of hemicellulose sugars and the technology involved in their conversion to valuable fuels and chemicals has recently been an area of intensive research [53]. Several approaches involving expression of fungal acetyl esterases and coumaroyl transferase have already been investigated regarding the degree of acetylation and substitution with ferulic acid esters [41 and references therein].

New approaches for the modification of xylan content and structure are now gaining attention that involve the use of tissue-specific promoters for the complementation of xylan mutants. Complementation of *Arabidopsis* xylan biosynthetic mutants with tissue-specific promoter driving expression of the wild type copy of the gene in the vessels but not in the interfascicular fibres showed decreased xylan content without impaired vessel function [46, 54]. These elegant studies demonstrate the power of synthetic biology in designing plant cell walls with desirable characteristics.

4 Lignin composition and biosynthesis

Lignin is a heterogeneous polymer of aromatic alcohols known as monolignols and is an integral part of SCW. Monolignols are synthesized in the cytoplasm, transported via ABC transporters to the apoplast [55] (Fig. 2A) and are polymerized via ether and C–C bonds. The lignification is achieved by the cross-linking of three primary hydroxycinnamyl alcohol monomers (*p*-coumaryl, coniferyl, and sinapyl) via radical–radical coupling generated by oxidative enzymes [56]. The spatial control of oxidative enzyme localization was shown to be important for lignification: a recent study demonstrated that the specific delivery of laccases control the lignification pattern. Although monolignol transporters display uniform localization, laccases are restricted to secondary cell walls throughout protoxylem tracheary element differentiation [57].

Despite the relevance of lignin in plant physiology and biofuel production, many of the aspects concerning its biosynthesis and patterned deposition are now starting to be understood. For example, it was recently demonstrated that during protoxylem formation, monolignols are mobile and that the targeting of laccases (LAC4 and LAC7 in *Arabidopsis thaliana*) to secondary cell walls dictates the deposition of lignin [57]. Lignin structure is very complex and present lignin models do not indicate any specific arrangement of monomeric units. A complex transcriptional network controls the xylem cell fate by determining the correct polarization of vascular cell types (xylem, phloem; extensively reviewed in [7]). The network also controls the expression of cell wall-related genes involved in the deposition of cellulose, hemicelluloses, and lignin [58]. There is a hierarchy in the transcriptional regulation acting on secondary cell wall-related genes: the first level of regulation involves master regulators belonging to the family NAC (**N**AM, **A**TAF1/2, and **C**UC2) that in turn regulate the second-level MYB transcription factors. Recently it was discovered that MYB46 transcription factor functions as a central and direct regulator of the genes involved in the biosynthesis of all three major (cellulose, hemicelluloses, and lignin) secondary wall components by binding to the promoters [59].

Enzyme-catalyzed oxidative coupling of phenolic compounds is a major reaction in the formation of intermediates for the biosynthesis of lignins [56, 60]. However, the control of regio- and stereospecificity remains puzzling [61]. It is now emerging that the biosynthesis of lignin at an early stage of formation may be guided by dirigent proteins [62, 63] first discovered in *Forsythia intermedia*, where they were found to direct the stereoselective biosynthesis of (+)-pinoresinol from coniferyl alcohol monomers (Supporting Information, Fig. S1) [62]. Dirigent proteins are extracellular glycoproteins with high β-strand content and have been found in all land plants investigated to date [e.g. [64, 65]]. Recently the first X-ray structure of a dirigent protein has been released and its ability to act as template in order to capture and orientate radicals resulting in radical–radical dimerization reactions is beginning to be understood [66]. The proposed structure is trimeric where all three substrate binding sites are far apart from each other and do not interact with one another, however the detailed mechanism of its action still requires further investigation. In the proposed mechanistic model, two radicals are bound by a homodimeric dirigent protein with the first radical binding reversibly, followed by an irreversible binding of the second, thereby initiating the formation of a C–C bond (Supporting Information, Fig. S1, [62, 66]).

Dirigent proteins lack their own catalytic activity, but direct the regio- and stereospecificity during lignan dimer biosynthesis, resulting in one highly enriched stereoisomer. Free radicals are generated from the substrate by the action of oxidative enzymes such as laccase and peroxidases, followed by the formation of a covalent C–C or C–O–C bond [67]. In the presence of conjugated double bond systems, radical–radical coupling can lead to the generation of a heterogeneous mixture of multiple products. The formation of side products or incorrect stereoisomers is a waste of energy and resources.When a dirigent protein is present during the reaction, one stereoisomer of a particular compound is highly enriched. Recent results indicate that dirigent domain containing proteins play a vital role in the correct formation of the extracellular lignin-based Casparian strip in endodermal cells of *A. thaliana* roots where its absence results in disorganized and defective lignin bands [63].

Over the years various hypothetical models of lignin molecular structures have been proposed based on random coupling theory and template replication model [61, 68, 69]. The coupling model proposes that monolignol units react endwise with the growing polymer with random linkages, whereas the template model suggests that polymerization is directed by sites on a template that defines lignin configuration [62, 69]. A well-defined model for a template process has been developed, that describes how a lignin primary structure is replicated by briefly placing lignol radicals about to undergo coupling on a double-stranded lignin template [69]. However further concrete experimental

evidence is required for such a model to gain widespread acceptance.

5 Enzymology of lignin formation

It is generally recognized that monolignols are derived from phenylalanine via a series of enzymatic reactions, catalyzed by the following enzymes: phenylalanine ammonialyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate coenzyme A ligase (4CL), ferulate 5-hydroxylase (F5H), *p*-coumarate3-hydroxylase (C3H), *p*-hydroxycinnamoyl-CoA:quinate/shikimate hydroxycinnamoyl transferase (HCT), caffeoyl-CoA *O*-methyltransferase (CCoAOMT), cinnamoyl-CoA reductase (CCR), caffeic acid *O*-methyltransferase (COMT), and cinnamyl alcohol dehydrogenase (CAD). It is noteworthy that in plants these genes are encoded by multigene families [70, 71]. This is not surprising, since plant genes show redundancy, especially those involved in secondary metabolism and ensure metabolic plasticity under unfavorable environmental conditions. The monomeric units are polymerized by radical–radical coupling when peroxidases (lignin-peroxidase, LiP; manganese-peroxidase, MnP; hybrid LiP-MnP versatile-peroxidase, VP) or laccase (benzenediol:oxygen oxidoreductase) convert monolignols to free-radicals [72]. Hydrogen peroxide required by peroxidases is produced by the action of superoxide dismutase, aryl-alcohol oxidase, and NADPH oxidases, [73].

CCoAOMT (EC 2.1.1.104) is a key enzyme in lignin biosynthesis in plants and its overexpression in transgenic plants results in enhanced plant height and silique length with concomitant increase in lignin content (20.4 -21.3%) compared to the control plants (17.56%) [74]. These molecular studies are vital if the fibre quality of economically important crops, such as jute, is the target of improvement. For example, in order to reduce lignin content in general, the CCoAOMT1 gene needs to be inhibited. Similarly CAD (EC 1.1.1.195), which catalyses the last step in monolignol synthesis, is a key enzyme involved in lignin biosynthesis and has a major role in the genetic regulation of lignin production [75].

6 Enzymology of lignin degradation

Lignin degradation is a very complex phenomenon requiring the concerted action of many hydrolytic and oxidative enzymes (Supporting Information, Fig. S2), as well as accessory proteins [76]. White-rot fungi secrete four major enzymes namely class II peroxidases, e.g. lignin peroxidase (LiP, EC 1.11.1.14), manganese peroxidase (MnP, EC 1.11.1.13), LiP-MnP hybrid versatile peroxidase (VP, EC 1.11.1.16, Supporting Information, Fig. S2) and an oxidoreductase, such as laccase (EC 1.10.3.2), all of which synergistically help degrade lignin [72, 76, 77]. The lignin degradation process is further improved by the cooperative action of several accessory enzymes such as glyoxal oxidase (EC 1.1.3.13), aryl-alcohol oxidase (EC 1.1.3.7), pyranose 2-oxidase (EC 1.1.3.10), cellobiose dehydrogenase, or cellobiose/quinone oxidoreductase (EC 1.1.99.18) [78]. LiP has a high-redox potential ($E_o \sim 1.2$ V at pH 3) compared to other peroxidases (horseradish, $E_o \sim 0.95$ V at pH 6.3) and oxidases (laccase, $E_o \sim 0.79$ V at pH 5.5). This allows the oxidation of nonphenolic recalcitrant aromatic substrates with concomitant cleavage of C–C and C–O–C bonds. On the other hand, MnP oxidizes Mn^{2+} to Mn^{3+} (E_o of only 0.8 V at pH 4.5), which in turn extracts an electron from the low redox substrates to generate free radicals. Whereas the bulky lignin substrate is oxidized at the surface of LiP enzyme, Mn^{3+}-dicarboxylic acid complex readily dissociates from the enzyme and penetrates deep inside the lignin molecule to bring about its oxidative degradation [72]. It has been proposed that LiP uses small molecules, such as veratryl alcohol as diffusible oxidants; however degradation of humic acid was not found to be enhanced in the presence of veratryl alcohol [79].

Hybrid VP (Supporting Information, Fig. S2), which combines the activities of both LiP and MnP continues to divulge exciting features regarding its allosteric behavior [72, 79, 80]. MnP activity in hybrid VP was found to be allosterically enhanced by Mn^{2+} [80]. Manganese is accumulated by plants and is present at high concentrations in decaying wood and fungal extracellular material that is involved in the degradation of lignin. MnP in VP makes effective use of Mn^{2+} to afford efficient degradation of lignin [80 and references therein]. In contrast, LiP activity in VP was inhibited by high substrate concentrations [80]. Interestingly using isothermal titration calorimetry (iTC), VP showed biphasic sigmoidal kinetics (cooperative kinetics) and product inhibition on polymeric humic substances indicative of allosteric behavior [79]. Relieving peroxidases of substrate (including H_2O_2) inhibition and product inhibition is essential if efficient degradation of lignin from lignocelluosic biomass is to be achieved in an industrial context [81]. One possible way to overcome substrate and product inhibition of peroxidases is to immobilize enzymes on nano-magnetic particles that can be easily separated from the reaction products by using a magnet [76 and references therein, 82]. The most interesting part of the study was that VP showed synergistic activation when both LiP and MnP were simultaneously and equally active compared to when either MnP or LiP was active alone [79]. In view of the demand to identify biocatalysts that can improve the release of cellulose from lignocellulosic biomass for biofuels, consideration should be given to the application of synergistically activated VP which can combine the high redox activity of LiP, as well as the MnP catalyzed diffusible capability of Mn^{3+}-dicarboxylic acid complex [72, 79].

Laccases (phenol oxidases) are copper containing enzymes found in bacteria, fungi, and plants that polymerize monolignols into lignin. However, regulation of plant laccases remains poorly understood. Laccases are involved in wound response, lignin biosynthesis, pigment formation in spores, detoxification of phenolic compounds produced during lignin degradation, and lignin breakdown [72, 77]. Recent research showed that in transgenic plants, overexpression of microRNA (miRNAs) reduced lignin deposition with a concomitant decrease in the thickness of the secondary walls of vessels leading to the weakening of vascular tissues. Laccase gene expression regulated by miRNAs may be exploited for engineering lignocellulosic biomass with reduced lignin content [83]. Although laccases have mostly been isolated and characterized from plant and fungal sources, bacterial enzymes are gaining importance in biotechnological applications due to their higher stability [84].

Recently a novel peroxidase, DyP (from dye-decolorizing), has been identified in the genomes of fungi, bacteria and archaea [85], however the physiological function of this class of enzymes is still unclear. Unlike VP where both MnP and LiP activities are oxidative, DyPs are bifunctional enzymes displaying oxidative, as well as hydrolytic activity. In addition, these enzymes are able to oxidize a variety of organic compounds many of which are poorly converted by known peroxidases. Evidence has been accumulating, which demonstrates that DyPs play a key role in the degradation of lignin [77, 85].

7 Biology, bioengineering, and biotechnology of lignin modification and degradation

The structural heterogeneity and recalcitrance of lignin makes it difficult for cell wall biomass conversion to gain economic value at the industrial level. Nevertheless, the existence of microbial pathways metabolizing aromatic compounds enables to shunt them to central metabolic intermediates which are further metabolized to acetyl-CoA. This scheme is now opening new avenues for the full exploitation of lignin-derived chemicals and for the creation of biosourced materials. For example the bacterium *Pseudomonas putida* KT2440 was used to convert the aromatic compounds derived from lignin into medium chain-length polyhydroxyalkanoate (*mcl*PHA) that can be used as bioplastics [86].

Surprisingly, the expression of monolignol biosynthesis genes is not always associated with the existence of the lignin polymer because a wide range of nonlignin products (hydroxycinnamic acids, phenol esters and phenolamides, or lignans) are also produced by the cell for various other requirements, such as water conduction, mechanical support, and defense of the plant [73]. This situation stresses the necessity for the stringent regulation of the lignification process. Metabolic engineering can be employed to reduce and/or modify natural lignin structure. A recent excellent study has shown the great potential of redesigning lignin structure by introducing chemically labile ester linkages (monolignols-ferulate conjugates) via the transferase gene in the xylem of poplar trees [87]. As a result, the cell wall of poplar trees is more easily digested after mild alkali pretreatment. Similarly, the downregulation of COMT (EC 2.1.1.68) and CCR (EC 1.2.1.44) enzymes have also shown very interesting results [88 and references therein]. Lower levels of COMT in switchgrass trigger moderate lignin reduction, along with a decrease in the syringyl and guaiacyl units (S/G) ratio. This results in an increase in ethanol yield with concomitant lower requirements of enzymes during simultaneous saccharification and fermentation [89]. However, downregulation of the CCR enzyme (that results in red coloration of the xylem due to the incorporation of ferulic acid) in poplar causes biomass yield penalties. During field trials in France and Belgium, biomass yields were reduced by 16%–24% for two different transgenic lines. In addition, there was a decrease in height (17%–27 %) and stem diameter (21%) for those lines showing a homogeneous red coloration of the xylem [90]. Another possible way to lower lignin content is by blocking the free parahydroxyl groups in monolignols responsible for the creation of lignin subunits via oxidative coupling (notably 4-*O*-methylation of monolignols). This showed no detrimental effect on transport across the membrane or on growth and development of the plant [91].

The production of novel and improved lignin structures in crops for biomaterial or bioethanol production is a current topic of intense research [60, 92]. Renewable biofuel production from lignocellulosic biomass is regarded as the most practicable choice for fossil fuel replacements, as these raw materials do not compete with food or feed crops [81].

Recent investigation into the consequences of lignin perturbations in *Arabidopsis* mutants highlighted that our knowledge of this complex process is far from perfect and that modifications in the lignin structure can be accompanied by unexpected consequences [93]. As previously mentioned, attempts to alter lignin biosynthesis frequently causes dwarfism or developmental abnormalities in transgenic plants resulting in low yield. The mechanisms that underlie this phenomenon are poorly understood and constitute another topic of current research [94]. The study by Gallego-Giraldo and colleagues [95] on *A. thaliana* and alfalfa showed a connection between impaired growth of the transgenic plants and salicylic acid (SA) biosynthesis. Blocking or redirecting SA synthesis to catechol, resulted in the restoration of normal plant growth. However, recent studies on the *Arabidopsis ref8* mutant clarified that depletion of SA does not rescue impaired growth [96] and that actually disruption of the Mediator Complex Subunits (MED5a and b) restores normal growth [97]. The latter study shows the importance of exploring perturbations in the cell wall transcriptional and signaling pathways as an avenue for engineering lignin deposition in plants.

Other promising approaches for the manipulation of lignin content without impairing the integrity of water transport tissues include the expression of miRNAs targeting enzymes that are involved in the phenylpropanoid pathway and are under the control of promoters driving expression in specific tissues [98].

Cellulose is hydrolyzed by cellulolytic enzymes into simple hexose sugars, which are fermented to ethanol by microbes. However, cellulose fibres are cross-linked in the plant cell walls with hemicellulose, along with the hydrophobic network of lignin (Fig. 4) that hinders the extraction of fermentable sugars from biomass. This has been one of the key obstacles in the development of cost-effective biofuel technologies [99, 100]. Current findings into the enzymatic degradation of lignocellulosic biomass by the termite gut flora and their associated enzymes suggest another promising avenue for the efficient industrial conversion of lignocellulose into simpler products and the production of biofuels [101]. A recent study has shed light on the wood digesting strategy of the so-called "termite of the sea," i.e. the marine bivalve *Bankia setacea* (a.k.a shipworm) [102] that used to cause great damages to ancient Greek and Roman wooden ships. The organism hosts, in specialized cells of its gills, a bacterial community responsible for the production of ligninolytic enzymes that are transported from the gills to the gut [102]. Interestingly, this wood-degrading system that rely on the distal production of ligninolytic enzymes is composed of only a small set (namely glycosyl hydrolases from families 5, 6, 9, 10, 11, 45, 53; carbohydrate esterases from families 1, 3, 4, 6, 15, and AA10) of enzymes predicted to be encoded by the endosymbiont genomes. These results are very important to

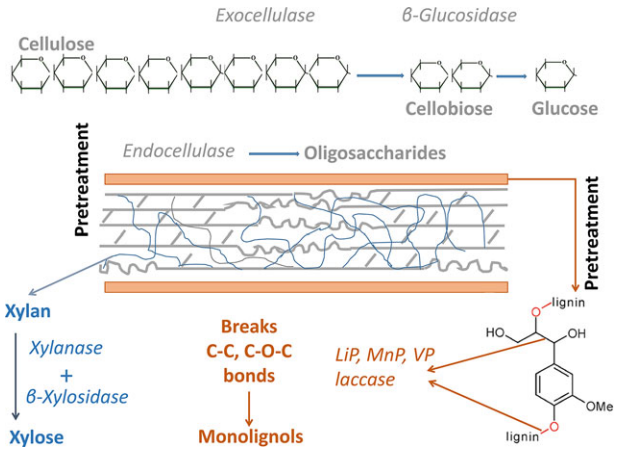

Figure 4. The enzymatic depolymerization of lignocellulosic biomass. Middle: lateral view of the plant cell wall showing cellulose fibres (grey). Crystalline cellulose fibres are depicted as straight and amorphous cellulose as wavy lines. Hemicellulose (blue) is shown embedded with cellulose fibres and surrounded by an impervious lignin matrix (brown). Various enzymes that can depolymerize these polymers are also shown along with their degradation products. Pretreatment is also indicated, which makes cellulose hydrolysis more efficient for cellulolytic enzymes by removing hemicellulosic and lignin matrix.

understand the minimal enzymatic requirement to achieve wood degradation [102].

8 Industrial applications of lignocellulose: The example of fibre crops

Lignocellulose is a rich and sustainable source of chemicals, fermentable sugars, and biopolymers that can lessen our dependence on petroleum-derived products. Plant biomass is indeed an important feedstock for the supply of raw material, e.g. cellulosic and woody fibres that can be used for the creation of "green" composites. Plant-sourced biocomposites designed by employing plant fibres as reinforcement and substitutes of glass fibres, are gaining a lot of attention because these are renewable, cheaper and do not raise health-related issues (irritation of skin/respiratory system). In this respect fast growing nonwoody species, like fibre crops are gaining considerable importance as these can provide a high amount of biomass in a relatively short period of time. For example hemp, *C. sativa* L., is estimated to yield more than 24 tons of biomass per hectare [103] and has a typical growth cycle of 100–120 days. These plants have stem tissues with contrasting lignification patterns and, consequently, different physicochemical properties. The core of the stem is lignified with short libriform cells (a.k.a "hurds" or "shivs"; 0.2–0.6 mm in hemp), while the cortex harbors long extraxylary fibres (the so-called "bast fibres" with a G-layer, see Section 1; primary bast fibres can be 5–60 mm in hemp) rich in crystalline cellulose and poor in lignin (Fig. 5); [104]. Both types of fibres are used for the creation of plant-sourced biomaterials, such as biocomposites from bast fibres and insulation material from hemp shivs. In addition, bast fibres are also used in textile applications. To

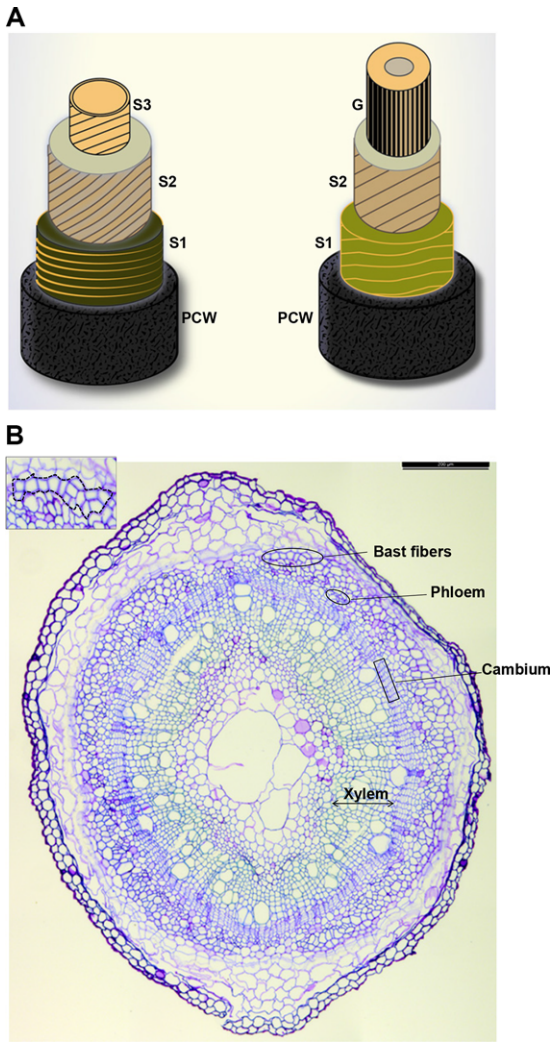

Figure 5. Organization of tissues in the fibre crop *Cannabis sativa* L. (A) Cartoon depicting the differences between woody fibres (left) and bast-fibres (right). G, gelatinous layer; S1-3, secondary wall layer; PCW, primary cell wall. (B) Cross section of hemp hypocotyls (3 weeks after sowing) showing the bast-fibres (magnified and highlighted by the dotted area in the inset picture), phloem, cambium, and xylem.

illustrate different applications of plant biomass, two examples will be briefly discussed: the use of hemp fibres for biocomposites and construction materials.

Hemp bast fibres are finding wide applications as reinforcement material in the polymer composite industry (e.g. for the creation of bioplastics in the automotive industry). Hemp fibres are cheaper than fiberglass and, importantly, they do not raise health-related issues [105]. Moreover they are recyclable, thus prompting no environmental concerns and are strong and resistant to decay [106]. The drawback of plant fibres for biocomposites is their hygroscopic nature leading to water absorption and changes in the mechanical properties of the biocomposite (micro-cracks due to fibre swelling and loss of interactions between the fibres and the matrix) [107 and references therein]

Hemp also provides woody fibres, the hurds or shivs that are used in the construction sector for the production of a concrete-like material. Hemp shivs are reported to be rich in silica (SiO_2) [108, 109] which in contact with lime, triggers a mineralization process leading to the formation of a lightweight stone-like material. Additionally, the presence of silica makes the biocomposite resistant to termite and rodent attacks.

9 Concluding remarks

Huge efforts need to be directed toward developments in cell wall engineering and downstream processing of plant biomass to ensure more efficient strategies for a full exploitation of this abundant natural bioresource. Hereafter is a catalogue of the major efforts that the scientific community has to undertake to rapidly progress in this research field.

9.1 Study of co-expressed gene networks

Studies elucidating co-expression networks of genes involved in SCW formation can identify key new factors. This approach can help in the identification of factors co-regulated in response to exogenous stresses. Recently a gene regulatory study focused on SCW synthesis in *A. thaliana* generated a consistent model predicting gene perturbation at the tissue level induced by several stresses (pH stress, sulfur, and iron derivation, salt stress) and nitrogen influx [110].

9.2 Enzyme resurrection

An extremely exciting branch of research in biotechnology is "enzyme resurrection," i.e. the recreation of ancient generalist enzymes that showed higher catalytic promiscuity and stability [111 and references therein]. This requires a bioinformatics-supported phylogenetic approach and is based on the premise that primitive cells were once equipped with a much more promiscuous and thermostable set of enzymes as compared to recent ones. [111, 112]. As discussed by Alcalde [111], the application of this approach to ligninases can rescue enzymes with broader substrate activities and/or stability that can be further optimized by adaptive evolution.

9.3 Transcriptional control of SCW synthesis

The study of the transcriptional control of SCW biosynthesis is challenging, but it can provide vital information to improve the quality and quantity of cell wall components. For example, synthetic biology can provide the means to rewire the cell network responsible for SCW deposition and achieve decreased lignin deposition, without impairing the integrity of the vessels [54]. A promoter swapping strategy was adopted by Yang and coworkers [54] to disconnect a key lignin deposition enzyme (C4H) from the fibre regulatory network and redirect its expression solely to the vessel network, thereby achieving reduced lignin content while preserving the vessel functionality. Similarly, they expressed the transcription factor NST1, responsible for the deposition of SCW, under the control of the IRX8 promoter (IRX8 is a *CesA* participating in SCW synthesis) to create an artificial positive feedback loop. NST1 binds to the IRX8 promoter to induce cellulose deposition. In their engineered set-up, the authors achieved a further expression of the transcription factor through positive feedback (NST1 auto-activates its own transcription) [54]. In order to achieve this goal, a solid knowledge of the promoter-transcription factor interaction is necessary.

9.4 Role of dirigent proteins in the synthesis of lignins

Findings based on a recently solved X-ray structure of a dirigent protein [66] and its role in the precise formation of lignin-based Casparian strips [63] suggests that free-radical coupling reactions are controlled by these "guiding" proteins [62]. However, further concrete mechanistic evidence is needed to displace the currently held view that the polymerization step in lignans and/or lignins is entirely under chemical control [113]. It is noteworthy that further experimental work has potential to find interesting biotechnological applications in the organic synthesis of stereo-specific pharmaceuticals by dirigent proteins [114].

9.5 Protein engineering for improvement of cell wall degrading enzymes

Further efforts in protein engineering are needed for improving enzyme properties to efficiently and cost-effectively degrade cell wall components necessary for biotechnological applications. Table 1 summarizes some recent examples of cellulases, xylanase, laccases, and peroxidases that have been genetically improved for high activity, enhanced stability, and reduced product inhibition. Readers are referred to a recently published excellent review on the genetic engineering of laccases for improvement in production, activity and stability [115]. Considering cellulases, deglycosylation of exo-cellulase results in enhanced activity due to its reduced binding to lignin whereas other amino acid replacements increase activity by relieving glucose (product) inhibition (Table 1). Introducing additional electrostatic interactions in endo-cellulases enhanced stability, while fusing cellulose-binding domain (CBD) with the catalytic domain simultaneously improved activity on insoluble crystalline substrates and thermostability (Table 1). Engineering stabilization mutations identified from computational methods, consensus design, and chimaera studies created the most stable fungal endo-cellulase reported to date, which was 17°C higher than the wild-type enzyme from *Hypocrea jecorina* [116]. A formulation of highly active and stable endo- and exo-cellulases showed three-fold higher productivity on crystalline cellulose due to synergy between both cellulases. Similarly, β-glucosidases have been engineered that simultaneously show improved activity and stability whereas the fusion of β-glucosidase with an endo-cellulase resulted in enhanced glucose production and reduced cellobiose (product) accumulation (Table 1). Based on synergy between all three cellulases (exo-, endo-cellulases and β-glucosidase) and reduction of product inhibition due to the removal of cellobiose by β-glucosidase, a complete genetically modified cellulose-degrading system that

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Table 1. Engineering improvements in the catalytic properties of enzymes involved in lignocellulose degradation.

Enzyme: Organism	Modifications	Catalytic properties	Remarks/causes	Reference
Exo-cellulase:				
T. reesei (Cel6A, Cel7A)	All R to E and S to T mutated in the linker region.	Increased hydrolytic activity on lignocellulosic substrate.	Reduced binding of the deglycosylated linker to lignin.	[118]
T. reesei (Cel6A)	Y103, L136, S186, G365, R410 to other residues.	Enhanced cellulose hydrolysis.	2 to 7-folds reduction in glucose inhibition.	[119]
P. funiculosum	A196S	Productivity of cellulose hydrolysis doubled from 12% to 25% after 120 h at 38°C. T_m (67.5→66.5°C)	Additional glycosylation slightly reduced stability but increased activity.	[120]
Endo-cellulase:				
C. thermocellum (Ct)	S329G/S269P/H194G	T_{opt} (75→85°C) $t_{1/2}$ (6→60 min: 86°C) Activity (16→19U mg^{-1}: 60°C)	Additional seven electrostatic interactions on the surface of active-site cleft.	[121]
Cryptococcus sp	CBD from *T. reesei* exo-cellulase fused to the C-terminal end of endo-cellulase from *Cryptococcus*	Activity: Sigmacell (0→2.3 U mg^{-1}) Activity: Avicel (0→0.5 U mg^{-1})	Activity on insoluble cellulosic substrates was dramatically enhanced due to increased binding of EG to the substrate.	[122]
B. amyloliquefaciens	E289V	Activity (0.7→5.6 U mg^{-1}: 50°C)	Eight-folds activity increased with a single amino acid change in the catalytic domain.	[123]
Cel5A from metagenomic library (vermicompost)	Error-prone PCR mutagenesis +	$t_{1/2}$ (1→seven-fold increase: 65°C) Activity, FP (0.4→2.9 U μmol^{-1})	The GM endo-cellulase fused with CBM had both seven-fold higher thermostability and activity on crystalline cellulose	[124]
CBD: *S. degradans*	Fusion of Cel5A with CBD	Activity, Avicel (0.2→1.4 U μmol^{-1})		
Engineered cellulase mixture:				
Endo-cellulase (Cel5A) +	Surface + core mutations that retained 100% activity and imparted thermostability by a combination of chimera-formation, foldX, consensus, α-helical dipole, backbone stability and improved core packing mechanisms.	Opt-Cel5A: T_{opt} (64→81°C) Activity on Avicel (1.5-fold higher) 3C6P-Cel6A: T_{opt} (65→75°C)	Individually engineered endo- and exo-cellulases showed higher activity and stability.	[116]
Exo-cellulases (Cel6A) +		Activity on Avicel (1.6-fold higher) TS8-Cel7A: T_{opt} (55→65°C) Activity on Avicel (1.5-fold excess)	T-PRIMED, showed three-folds higher productivity on crystalline cellulose relative to wild-type mix at 70°C due to synergistic activation between endo-and exo-cellulases.	
Exo-cellulase (Cel7A) *(T. reesei)*		T-PRIMED (mix of three engineered cellulases) showed 2.5 to 3-fold higher productivity on avicel and rice-straw, respectively, at 70°C.		
β-glucosidase:				
T. fusca and P. polymyxa	Combination of gene shuffling from *T. fusca* and *P. polymxyxa*, site-saturation and site-directed mutagenesis. L444Y/G447S/A433V	Both activity and stability were enhanced simultaneously. $t_{1/2}$ (12→1732 min: 61°C) k_{cat} (2.8→5.4 s^{-1}: 37°C)	L444Y/G447S confers extra H-bonding. A433V results in an increased sidechain volume, increased hydrophobicity and packing efficiency. Reduced distance (5.84-5.29 Å) between V433 and catalytic Glu388.	[117]

(*Continued*)

Table 1. Continued.

Enzyme: Organism	Modifications	Catalytic properties	Remarks/causes	Reference
Endo-cellulase (CtCD) + β-glucosidase (CcBG)	CtCD CcBG CtCD-CcBG fused enzyme CcBG-CtCD fused enzyme	247 U μmol^{-1} (cellulose), T_m, 71°C 3395 U μmol^{-1} (salicin), T_m, 55°C 477 U μmol^{-1} (cellulose), T_m, 66°C 205 U μmol^{-1} (cellulose)	The fused hybrid enzyme showed two-fold higher glucose production and three-fold reduced cellobiose accumulation relative to their mixture.	[125]
Complete cellulase system: exo-cellulase, cbhA + endo-cellulase, cenA + (*C. fimi*)	Co-expression of all three cellulolytic enzymes in *E. coli.*	cbhA: 4 nmol glucose (gluc) mg^{-1} cenA: 13 nmol gluc mg^{-1} bgl: 0 nmol gluc mg^{-1} cbhA-cenA-bgl (wild-type): 134 nmol gluc mg^{-1}	Co-engineered cellulase system produced eight-fold higher glucose then separate enzymes due to synergistic activation.	[126]
β-glucosidase, BG1 *(T. reesei)*	Further directed-evolution of co-engineered cellulase system. V615A(cbhA)T236A/P299A/L387P (cenA)P172L/K205R (bgl)	↓(assayed at 40°C with filter paper) cbhA-cenA-bgl (evolved mutant): 357 nmol gluc mg^{-1}	The GM cellulolytic enzymes produced further 2.7-fold higher gluc.	[127]
Xylanase:				
Streptomyces sp9	V81P/G82E	k_{cat} (790→203 s^{-1}: 70°C) T_{opt} (65→90°C)	Enhanced rigidity due to additional H-bonds near the active-site.	[128]
		T_m (68→75°C) $t_{1/2}$ (9→94 min: 70°C)	Enhanced rigidity of the hinge region.	[129]
B. circulans	N141V	V_{max} (67→131 mM min^{-1}) T_m (47→46°C)		
Versatile peroxidase:				
P. eryngii	M247L A77E, I81L, A77E/A79S/I81L	Increased H_2O_2 tolerance and activity	Replacement of oxidizable Met and residues close to H_2O_2 binding site.	[130]
P. eryngii	E, K and/or P mutated to G	Increased k_{cat} and reduced K_m on many phenolic substrates.	Increased activity due to removal of bulky sidechains from the active-site tunnel.	[131]
Laccase:	E188R	k_{cat} (20→60 s^{-1}: 25°C) 100 % activation (65°C, 90 min)	New cation-pi interaction in the inter-domain surface loop increases packing efficiency and structural compactness.	[132]
Bacillus HR03	E188K	k_{cat} (20→14 s^{-1}: 25°C) 500% activation (65°C, 90 min)		

T_m, melting temperature; T_{opt}, optimum temperature; $t_{1/2}$, half-life of thermal inactivation; *gluc, glucose;* →, change in activity and stability from wild-type (WT) to mutant; CBD, cellulose binding domain; GM, genetically modified; CD, catalytic domain; EG, endo-glucanase; *S. degradans, Saccharophagus degradans*; *B. amylliquefaciens, Bacillus amyloliquefaciens; C. thermocellum, Clostridium thermocellum*; Cc, *Clostridium cellulovorans; T. reesei, Trichoderma reesei*; *P. funiculosum, Penicillium funiculosum; T. fusca, Thermobifida fusca*; *P. polymyxa, Paenibacillus polymyxa; C. fimi, Cellulomonas fimi; B. circulans, Bacillus circulans*; *P. eryngii, Pleurotus eryngii.*

showed a 10-fold increase in glucose production was engineered (Table 1). It is noteworthy that hydrolysis of cellobiose (produced by exo- and endo-cellulases) to glucose becomes limiting due to thermolability of β-glucosidase from *Trichoderma reesei*. To overcome this limitation, genetically engineered thermostable β-glucosidase from *T. fusca* and *Paenibacillus polymyxa* [117] can be employed in lieu of *T. reesei* enzyme (Table 1). Collectively these results imply that amino acid replacements generated by various genetic modification methods, which stabilize the enzyme structure, enhance activity or relieve product inhibition, may be combined. This can be achieved by developing enzyme formulations or fusing lignocellulose-degrading enzymes, thereby synergistically enhancing the deconstruction of biomass at high temperatures (Table 1).

Practical application

This review is an up-to-date summary of the current knowledge of the mechanisms regulating the synthesis and degradation of lignocellulose components. In particular, the review is focused on three main components of plant cell wall, i.e. cellulose, xylan, and lignin. We have attempted to approach the topic from a wider perspective by examining the structural, genetic and enzymological aspects of biosynthesis and degradation of cellulose, xylan, and lignin. Examples regarding the industrial use of lignocellulose are provided, by focusing on fast growing herbaceous species, such as the fibre crop *Cannabis sativa*. The review ends by proposing future avenues of research with the purpose of stimulating the scientific and business community engaged in the utilization of lignocellulosic biomass.

The conceiving of this review is linked to activities conducted within the COST FP1105 and COST FP1106 actions. GG gratefully acknowledges the Austrian Science Fund (FWF; http://www.fwf.ac.at/en/) Grant No. M1315. GG and JFH wish to thank the support by the Fonds National de la Recherche, Luxembourg, (Project CANCAN C13/SR/5774202 and project CADWALL INTER/FWO/12/14). Personal support of KSS by KFUPM is acknowledged. The authors wish to thank Dr. Fuad Bahram for drawing the picture appearing in Fig. 5(A) and Laurent Solinhac for providing the microscopy picture in Fig. 5(B).

The authors have declared no conflict of interest.

10 References

[1] Mellerowicz, E. J., Gorshkova, T. A., Tensional stress generation in gelatinous fibers: A review and possible mechanism based on cell-wall structure and composition. *J. Exp. Bot.* 2012, *63*, 551–565.

[2] Guerriero, G., Sergeant, K., Hausman, J. F., Integrated -omics: A powerful approach to understanding the heterogeneous lignification of fiber crops. *Int. J. Mol. Sci.* 2013, *4*, 10958–10978.

[3] Fry, S. C., Phenolic components of the primary cell wall. Feruloylated disaccharides of D-galactose and L-arabinose from spinach polysaccharide. *Biochem. J.* 1982, *203*, 493–504.

[4] Wi, S. G., Singh, A. P., Lee, K. H., Kim, Y. S., The pattern of distribution of pectin, peroxidase and lignin in the middle lamella of secondary xylem fibres in alfalfa (*Medicago sativa*). *Ann. Bot.* 2005, *95*, 863–868.

[5] Christiernin, M., Ohlsson, A. B., Berglund, T., Henriksson, G., Lignin isolated from primary walls of hybrid aspen cell cultures indicates significant differences in lignin structure between primary and secondary cell wall. *Plant Physiol. Biochem.* 2005, *43*, 777–785.

[6] Hao, Z., Mohnen, D., A review of xylan and lignin biosynthesis: Foundation for studying *Arabidopsis* irregular xylem mutants with pleiotropic phenotypes. *Crit. Rev. Biochem. Mol. Biol.* 2014, *49*, 212–241.

[7] Guerriero, G., Sergeant, K., Hausman, J. F., Wood biosynthesis and typologies: A molecular rhapsody. *Tree Physiol.* 2014, *34*, 839–855.

[8] Parthasarathi, R., Bellesia, G., Chundawat, S. P., Dale, B. E. et al., Insights into hydrogen bonding and stacking interactions in cellulose. *J. Phys. Chem. A.* 2011, *115*, 14191–14202.

[9] Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S. et al., Biomass recalcitrance: Engineering plants and enzymes for biofuels production. *Science* 2007, *315*, 804–807. Erratum in: *Science* 2007, *316*, 982.

[10] Brown, R. M., Jr., The biosynthesis of cellulose. *J. Macromol. Sci., Pure Appl. Chem.* 1996, *33*, 1345–1373.

[11] Wightman, R., Turner, S., Trafficking of the cellulose synthase complex in developing xylem vessels. *Biochem. Soc. Trans.* 2010, *38*, 755–760.

[12] Bashline, L., Li, S., Gu, Y., The trafficking of the cellulose synthase complex in higher plants. *Ann. Bot.* 2014, *114*, 1059–1067.

[13] Crowell, E. F., Bischoff, V., Desprez, T., Rolland, A. et al., Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis*. *Plant Cell* 2009, *21*, 1141–1154.

[14] Paredez, A. R., Somerville, C. R., Ehrhardt, D. W., Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* 2006, *312*, 1491–1495.

[15] Bowling, A. J., Brown, R. M., Jr., The cytoplasmic domain of the cellulose-synthesizing complex in vascular plants. *Protoplasma* 2008, *233*, 115–127.

[16] Slabaugh, E., Davis, J. K., Haigler, C. H., Yingling, Y. G. et al., Cellulose synthases: New insights from crystallography and modeling. *Trends Plant Sci.* 2014, *19*, 99–106.

[17] Sethaphong, L., Haigler, C. H., Kubicki, J. D., Zimmer, J. et al., Tertiary model of a plant cellulose synthase. *Proc. Natl. Acad. Sci. U.S.A.* 2013, *110*, 7512–7517.

[18] Olek, A. T., Rayon, C., Makowski, L., Kim, H. R. et al., The structure of the catalytic domain of a plant cellulose synthase and its assembly into dimers. *Plant Cell* 2014, *22*, 2996–3009.

[19] Taylor, N. G., Howells, R. M., Huttly, A. K, Vickers, K. et al., Interactions among three distinct CesA proteins essential for cellulose synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 2003, *100*, 1450–1455.

[20] Persson, S., Paredez, A., Carroll, A., Palsdottir, H. et al., Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 2007, *104*, 15566–15571.

[21] Carroll, A., Mansoori, N., Li, S., Lei, L. et al., Complexes with mixed primary and secondary cellulose synthases are functional in *Arabidopsis* plants. *Plant Physiol.* 2012, *160*, 726–737.

[22] Gu, Y., Kaplinsky, N., Bringmann, M., Cobb, A. et al., Identification of a cellulose synthase-associated protein required for cellulose biosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* 2010, *107*, 12866–12871.

[23] Gu, Y., Somerville, C., Cellulose synthase interacting protein: A new factor in cellulose synthesis. *Plant Signal. Behav.* 2010, *5*, 1571–1574.

[24] Ruprecht, C., Mutwil, M., Saxe, F., Eder, M. et al., Large-scale co-expression approach to dissect secondary cell wall formation across plant species. *Front. Plant Sci.* 2011, *2*, 23.

[25] Lynd, L. R., Weimer, P. J., van Zyl, W. H., Pretorius, I. S., Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev.* 2002, *66*, 506–577.

[26] Lynd, L. R., van Zyl, W. H., McBride, J. E., Laser, M., Consolidated bioprocessing of cellulosic biomass: An update. *Curr. Opin. Biotechnol.* 2005, *16*, 577–583.

[27] Horn, S. J., Vaaje-Kolstad, G., Westereng, B., Eijsink, V. G., Novel enzymes for the degradation of cellulose. *Biotechnol. Biofuels* 2012, *5*, 45.

[28] Schubot, F. D., Kataeva, I. A., Chang, J., Shah, A. K. et al., Structural basis for the exocellulase activity of the cellobiohydrolase CbhA from *Clostridium thermocellum*. *Biochemistry* 2004, *43*, 1163–1170.

[29] Payne, C. M., Resch, M. G., Chen, L., Crowley, M. F. et al., Glycosylated linkers in multimodular lignocellulose-degrading enzymes dynamically bind to cellulose. *Proc. Natl. Acad. Sci. U.S.A.* 2013, *110*, 14646–14651.

[30] Vaaje-Kolstad, G., Westereng, B., Horn, S. J., Liu, Z. et al., An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. *Science* 2010, *330*, 219–222.

[31] Westereng, B., Ishida, T., Vaaje-Kolstad, G., Wu, M. et al., The putative endoglucanase PcGH61D from *Phanerochaete chrysosporium* is a metal-dependent oxidative enzyme that cleaves cellulose. *PLoS One* 2011, *6*, e27807.

[32] Phillips, C. M., Beeson, W. T., Cate, J. H., Marletta, M. A., Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by *Neurospora crassa*. *ACS Chem. Biol.* 2011, *6*, 1399–1406.

[33] Levasseur, A., Drula, E., Lombard, V., Coutinho, P. M. et al., Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. *Biotechnol. Biofuels* 2013, *6*, 41.

[34] Quinlan, R. J., Sweeney, M. D., Leggio L. L., Otten, H. et al., Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. *Proc. Natl. Acad. Sci. U.S.A.* 2011, *108*, 15079–15084.

[35] Beeson, W. T., Phillips, C. M., Cate, J. H. D., Marletta, M. A., Oxidative cleavage of cellulose by fungal copper-dependent polysaccharide monooxygenases. *J. Am. Chem. Soc.* 2012, *134*, 890–892.

[36] Isaksen, T., Westereng, B., Aachmann, F. L., Agger, J. W. et al., A C4-oxidizing lytic polysaccharide monooxygenase cleaving both cellulose and cello-oligosaccharides. *J. Biol. Chem.* 2014, *289*, 2632–2642.

[37] Agger, J. W., Isaksen, T., Várnai, A., Vidal-Melgosa, S. et al., Discovery of LPMO activity on hemicelluloses shows the importance of oxidative processes in plant cell wall degradation. *Proc. Natl. Acad. Sci. U.S.A.* 2014, *111*, 6287–6292.

[38] Forsberg, Z., Vaaje-Kolstad, G., Westereng, B., Bunæs, A. C. et al., Cleavage of cellulose by a CBM33 protein. *Prot. Sci.* 2011, *20*, 1479–1483.

[39] Franková, L., Fry, S. C., Biochemistry and physiological roles of enzymes that 'cut and paste' plant cell-wall polysaccharides. *J. Exp. Bot.* 2013, *64*, 3519–3550.

[40] Pauly, M., Gille, S., Liu, L., Mansoori N. et al., Hemicellulose biosynthesis. *Planta* 2013, *238*, 627–642.

[41] Rennie, E. A., Scheller, H. V., Xylan biosynthesis. *Curr. Opin. Biotechnol.* 2014, *26*, 100–107.

[42] Brown, D. M., Zhang, Z. N., Stephens, E., Dupree, P. et al., Characterization of IRX10 and IRX10-like reveals an essential role in glucuronoxylan biosynthesis in *Arabidopsis*. *Plant J.* 2009, *57*, 732–746.

[43] Wu, A-M., Hörnblad, E., Voxeur, A., Gerber, L. et al., Analysis of the *Arabidopsis* IRX9/IRX9-L and IRX14/IRX14-L pairs of glycosyltransferase genes reveals critical contributions to biosynthesis of the hemicelluloses glucuronoxylan. *Plant Physiol.* 2010, *153*, 542–554.

[44] Keppler, B. D., Showalter, A. M., IRX14 and IRX14-LIKE, two glycosyl transferases involved in glucuronoxylan biosynthesis and drought tolerance in *Arabidopsis*. *Mol. Plant* 2010, *3*, 834–841.

[45] Bromley, J. R., Busse-Wicher, M., Tryfona, T., Mortimer, J. C. et al., GUX1 and GUX2 glucuronyltransferases decorate distinct domains of glucuronoxylan with different substitution patterns. *Plant J.* 2013, *74*, 423–434.

[46] Petersen, P. D., Lau, J., Ebert, B., Yang, F. et al., Engineering of plants with improved properties as biofuels feedstocks by vessel-specific complementation of xylan biosynthesis mutants. *Biotechnol. Biofuels* 2012, *5*, 84.

[47] Busse-Wicher, M., Gomes, T. C., Tryfona, T., Nikolovski, N. et al., The pattern of xylan acetylation suggests xylan may interact with cellulose microfibrils as a two-fold helical screw in the secondary plant cell wall of *Arabidopsis thaliana*. *Plant J.* 2014, *79*, 492–506.

[48] Manabe, Y., Nafisi, M., Verhertbruggen, Y., Orfila, C. et al., Loss-of-function mutation of reduced wall acetylation2 in *Arabidopsis* leads to reduced cell wall acetylation and increased resistance to *Botrytis cinerea*. *Plant Physiol.* 2011, *155*, 1068–1078.

[49] Manabe, Y., Verhertbruggen, Y., Gille, S., Harholt, J. et al., RWA proteins play vital and distinct roles in cell wall O-acetylation in *Arabidopsis thaliana*. *Plant Physiol.* 2013, *163*, 1107–1117.

[50] Xiong, G., Cheng, K., Pauly, M., Xylan O-acetylation impacts xylem development and enzymatic recalcitrance as indicated by the *Arabidopsis* mutant tbl29. *Mol. Plant* 2013, *6*, 1373–1375.

[51] Urbanowicz, B. R., Peña, M. J., Ratnaparkhe, S., Avci, U. et al., 4-*O*-methylation of glucuronic acid in *Arabidopsis*

glucuronoxylan is catalyzed by a domain of unknown function family 579 protein. *Proc. Natl. Acad. Sci. U.S.A.* 2012, *109*, 14253–14258.

[52] Anders, N., Wilkinson, M. D., Lovegrove, A., Freeman, J. et al., Glycosyl transferases in family 61 mediate arabinofuranosyl transfer onto xylan in grasses. *Proc. Natl. Acad. Sci. U.S.A.* 2012, *109*, 989–993.

[53] Ji, X. J., Huang, H., Nie, Z. K., Qu, L. et al., Fuels and chemicals from hemicellulose sugars. *Adv. Biochem. Eng. Biotechnol.* 2012, *128*, 199–224.

[54] Yang, F., Mitra, P., Zhang, L., Prak, L. et al., Engineering secondary cell wall deposition in plants. *Plant Biotechnol. J.* 2013, *11*, 325–335.

[55] Miao, Y. C., Liu, C. J., ATP-binding cassette-like transporters are involved in the transport of lignin precursors across plasma and vacuolar membranes. *Proc. Natl. Acad. Sci. U.S.A.* 2010, *107*, 22728–22733.

[56] Boerjan, W., Ralph, J., Baucher, M., Lignin biosynthesis. *Annu. Rev. Plant Biol.* 2003, *54*, 519–546.

[57] Schuetz, M., Benske, A., Smith, R., Watanabe Y. et al., Laccases direct lignification in the discrete secondary cell wall domains of protoxylem. *Plant Physiol.* 2014, *166*, 798–807.

[58] Zhong, R., Ye, Z. H., Complexity of the transcriptional network controlling secondary wall biosynthesis. *Plant Sci.* 2014, *229*, 193–207.

[59] Kim, W. C., Kim, J. Y., Ko, J. H., Kang, H. et al., Identification of direct targets of transcription factor MYB46 provides insights into the transcriptional regulation of secondary wall biosynthesis. *Plant Mol. Biol.* 2014, *85*, 589–599.

[60] Vanholme, R., Morreel, K., Darrah, C., Oyarce, P. et al., Metabolic engineering of novel lignin in biomass crops. *New Phytol.* 2012, *196*, 978–1000.

[61] Hatfield, R., Vermerris, W., Lignin formation in plants. The dilemma of linkage specificity. *Plant Physiol.* 2001, *126*, 1351–1357.

[62] Davin, L. B., Lewis, N. G., Lignin primary structures and dirigent sites. *Curr. Opin. Biotechnol.* 2005, *16*, 407–415.

[63] Hosmani, P. S., Kamiya, T., Danku, J., Naseer, S. et al., Dirigent domain-containing protein is part of the machinery required for formation of the lignin-based Casparian strip in the root. *Proc. Natl. Acad. Sci. U.S.A.* 2013, *110*, 14498–14503.

[64] Li, Q., Chen, J., Xiao, Y., Di, P. et al., The dirigent multigene family in *Isatis indigotica*: Gene discovery and differential transcript abundance. *BMC Genomics* 2014, *15*, 388.

[65] Behr, M., Legay, S., Hausman, J.F., Guerriero, G., Analysis of cell wall-related genes in organs of *Medicago sativa* L. under different abiotic stresses. *Int. J. Mol. Sci.* 2015, *16*, 16104–16124.

[66] Kim, K. W., Smith, C. A., Daily, M. D., Cort, J. R. et al., Trimeric structure of (+)-pinoresinol-forming dirigent protein at 1.95 Å resolution with three isolated active sites. *J. Biol. Chem.* 2015, *290*, 1308–1318.

[67] Dashtban, M., Schraft, H., Syed, T. A., Qin, W., Fungal biodegradation and enzymatic modification of lignin. *Int. J. Biochem. Mol. Biol.* 2010, *1*, 36–50.

[68] Chen, Y-R., Sarkanen, S., Macromolecular lignin replication—a mechanistic working hypothesis. *Phytochem. Rev.* 2003, *2*, 235–255.

[69] Chen, Y-R., Sarkanen, S., Macromolecular replication during lignin biosynthesis. *Phytochemistry* 2010, *71*, 453–462.

[70] Humphreys,J. M., Chapple,C., Rewriting the lignin roadmap. *Curr. Opin. Plant Biol.* 2002, *5*, 224–229.

[71] Fraser, C. M., Chapple, C., The phenylpropanoid pathway in Arabidopsis. *Arabidopsis Book.* 2011, *9*, e0152.

[72] Wong, D. W. S., Structure and action mechanism of ligninolytic enzymes. *Appl. Biochem. Biotechnol.* 2009, *157*, 174–209.

[73] Wang, Y., Chantreau, M., Sibout, R., Hawkins, S., Plant cell wall lignification and monolignol metabolism. *Front. Plant Sci.* 2013, *4*, 220.

[74] Zhang, G., Zhang, Y., Xu, J., Niu, X. et al., The CCoAOMT1 gene from jute (*Corchorus capsularis* L.) is involved in lignin biosynthesis in *Arabidopsis thaliana*. *Gene* 2014, *546*, 398–402.

[75] Tang, R., Zhang, X. Q., Li, Y. H., Xie, X. M., Cloning and in silico analysis of a cinnamyl alcohol dehydrogenase gene in *Pennisetum purpureum*. *J. Genet.* 2014, *93*, 145–158.

[76] Guerriero, G., Hausman, J. F., Strauss, J., Ertan, H. et al., Destructuring plant biomass: Focus on fungal and extremophilic cell wall hydrolases. *Plant Sci.* 2015, *234*, 180–193.

[77] Abdel-Hamid, A. M., Solbiati, J. O., Cann, I. K., Insights into lignin degradation and its potential industrial applications. *Adv. Appl. Microbiol.* 2013, *82*, 1–28.

[78] Mester, T., Varela, E., Tien, M., Wood degradation by brown-rot and white-rot fungi, in: Kück, U., (Ed.), *The Mycota II: Genetics and Biotechnology* (2nd edn), Springer-Verlag, Berlin - Heidelberg 2004, pp. 355–365.

[79] Siddiqui, K. S., Ertan, H., Charlton, T., Poljak, A. et al., Versatile peroxidase degradation of humic substances: Use of isothermal titration calorimetry to assess kinetics, and applications to industrial wastes. *J. Biotechnol.* 2014, *178*, 1–11.

[80] Ertan, H., Siddiqui, K. S., Muenchhoff, J., Charlton, T. et al., Kinetic and thermodynamic characterization of the functional properties of a hybrid versatile peroxidase using isothermal titration calorimetry: Insight into manganese peroxidase activation and lignin peroxidase inhibition. *Biochimie* 2012, *94*, 1221–1231.

[81] Moreno, A. D., Ibarra, D., Alvira, P., Tomás-Pejó, E. et al., A review of biological delignification and detoxification methods for lignocellulosic bioethanol production. *Crit. Rev. Biotechnol.* 2015, *35*, 342–354.

[82] Netto, C. G. C. M., Toma, H. E., Andrade, L. H., Superparamagnetic nanoparticles as versatile carriers and supporting materials for enzymes. *J. Mol. Catal. B: Enzymatic* 2013, *85*, 71–92.

[83] Wang, C. Y., Zhang, S., Yu, Y., Luo, Y. C. et al., MiR397b regulates both lignin content and seed number in *Arabidopsis* via modulating a laccase involved in lignin biosynthesis. *Plant Biotechnol. J.* 2014, *12*, 1132–1142.

[84] Muthukumarasamy, N. P., Murugan, S., Production, purification and application of bacterial laccase: A review. *Biotechnology*, 2014, *13*, 196–205.

[85] Colpa, D. I., Fraaije, M. W., van Bloois, E., DyP-type peroxidases: A promising and versatile class of enzymes. *J. Ind. Microbiol. Biotechnol.* 2014, *41*, 1–7.

[86] Linger, J. G., Vardon, D. R., Guarnieri, M. T., Karp, E. M. et al., Lignin valorization through integrated biological funneling

and chemical catalysis. *Proc. Natl. Acad. Sci. U.S.A.* 2014, *111*, 12013–12018.

[87] Wilkerson, C. G., Mansfield, S. D., Lu, F., Withers, S. et al., Monolignol ferulate transferase introduces chemically labile linkages into the lignin backbone. *Science* 2014, *344*, 90–93.

[88] Liu, C. J., Cai, Y., Zhang, X., Gou, M. et al., Tailoring lignin biosynthesis for efficient and sustainable biofuel production. *Plant Biotechnol. J.* 2014, *12*, 1154–1162.

[89] Fu, C., Mielenz, J. R., Xiao, X., Ge, Y. et al., Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. *Proc. Natl. Acad. Sci. U.S.A.* 2011, *108*, 3803–3808.

[90] Van Acker, R., Leple, J. C., Aerts, D., Storme, V. et al., Improved saccharification and ethanol yield from field-grown transgenic poplar deficient in cinnamoyl-CoA reductase. *Proc. Natl. Acad. Sci. U.S.A.* 2014, *111*, 845–850.

[91] Zhang, K. W., Bhuiya, M. W., Pazo, J. R., Miao, Y. et al., An engineered monolignol 4-*O*-methyltransferase (MOMT4) represses lignin polymerization and confers novel metabolic capability in *Arabidopsis*. *Plant Cell* 2012, *24*, 3122–3139.

[92] Ragauskas, A. J., Beckham, G. T., Biddy, M. J., Chandra, R. et al., Lignin valorization: Improving lignin processing in the biorefinery. *Science* 2014, *344*, 1246843.

[93] Vanholme, R., Storme, V., Vanholme, B., Sundin, L. et al., A systems biology view of responses to lignin biosynthesis perturbations in *Arabidopsis*. *Plant Cell* 2012, *24*, 3506–3529.

[94] Bonawitz, N. D., Chapple, C., Can genetic engineering of lignin deposition be accomplished without an unacceptable yield penalty? *Curr. Opin. Biotechnol.* 2013, *24*, 336–343.

[95] Gallego-Giraldo, L., Escamilla-Trevino, L., Jackson, L. A., Dixon, R. A., Salicylic acid mediates the reduced growth of lignin down-regulated plants. *Proc. Natl. Acad. Sci. U.S.A.* 2011, *108*, 20814–20819.

[96] Kim, J. I., Ciesielski, P. N., Donohoe, B. S., Chapple, C. et al., Chemically induced conditional rescue of the reduced epidermal fluorescence mutant of Arabidopsis reveals rapid restoration of growth and selective turnover of secondary metabolite pools. *Plant Physiol.* 2014, *164*, 584–595.

[97] Bonawitz, N. D., Kim, J. I., Tobimatsu, Y., Ciesielski, P. N. et al., Disruption of Mediator rescues the stunted growth of a lignin-deficient *Arabidopsis* mutant. *Nature* 2014, *509*, 376–380.

[98] Smith, R. A., Schuetz, M., Roach, M., Mansfield, S. D. et al., Neighboring parenchyma cells contribute to *Arabidopsis* xylem lignification, while lignification of interfascicular fibers is cell autonomous. *Plant Cell* 2013, *25*, 3988–3999.

[99] Zeng, Y., Zhao, S., Yang, S., Ding, S. Y., Lignin plays a negative role in the biochemical process for producing lignocellulosic biofuels. *Curr. Opin. Biotechnol.* 2014, *27*, 38–45.

[100] Eudes, A., Liang, Y., Mitra, P., Loqué, D., Lignin bioengineering. *Curr. Opin. Biotechnol.* 2014, *26*, 189–198.

[101] Brune, A., Symbiotic digestion of lignocellulose in termite guts. *Nat. Rev. Microbiol.* 2014, *12*, 168–180.

[102] O'Connor, R. M., Fung, J. M., Sharp, K. H., Benner, J. S. et al., Gill bacteria enable a novel digestive strategy in a wood-feeding mollusk. *Proc. Natl. Acad. Sci. U.S.A.* 2014, *111*, E5096–E51104.

[103] Kolaříková, M., Ivanova, T., Havrland, B., Energy balance of briquettes made of hemp (*Cannabis sativa* L.) cultivars (Ferimon, Bialobrzeskie) from autumn harvest to produce heat for household use. In Engineering for Rural Development. Latvia University of Agriculture, Jelgava 23, Latvia 2013, pp. 504–508.

[104] Guerriero, G., Sergeant, K., Hausman, J. F., Integrated *-omics*: A powerful approach to understanding the heterogeneous lignification of fibre crops. *Int. J. Mol. Sci.* 2013, *14*, 10958–10978.

[105] Kilburn, K. H., Powers, D., Warshaw, R. H., Pulmonary effects of exposure to fine fibreglass: Irregular opacities and small airways obstruction. *Br. J. Ind. Med.* 1992, *49*, 714–720.

[106] Small, E., Marcus, D., Hemp: A new crop with new uses for North America. In: Janick, J., Whipkey, A., (eds.), *Trends in New Crops and New Uses*. ASHS Press, Alexandria, VA 2002, pp. 284–326.

[107] Célino, A., Fréour, S., Jacquemin, F., Casari, P., The hygroscopic behavior of plant fibers: A review. *Front. Chem.* 2013, *1*, 43.

[108] Grace, J. K., Termite response to agricultural fiber composites: Hemp. International Research Group on Wood Preservation, Stockholm, Sweden. 36th Annual Meeting Bangalore, 2005, India.

[109] Danenberg, J. M., Hemp Architecture. 1997 Catalyst Conference, University of Canberra. Hemp SA Inc., Australia.

[110] Taylor-Teeples, M., Lin, L., de Lucas, M., Turco, G. et al. An *Arabidopsis* gene regulatory network for secondary cell wall synthesis. *Nature* 2015, *517*, 571–575.

[111] Alcalde, M., Engineering the ligninolytic enzyme consortium. *Trends Biotechnol.* 2015, *33*, 155–162.

[112] Hobbs, J. K., Shepherd, C., Saul, D. J., Demetras, N. J. et al., On the origin and evolution of thermophily: Reconstruction of functional precambrian enzymes from ancestors of *Bacillus*. *Mol. Biol. Evol.* 2012, *29*, 825–835.

[113] Ralph, J., Brunow, G., Harris, P. J., Dixon, R. A. et al., Lignification: Are lignins biosynthesized via simple combinatorial chemistry or via proteinaceous control and template replication? Daayf, F., Lattanzio, V. (eds.), in: *Recent Advances in Polyphenol Research*. Blackwell Publishing Ltd., Singapore, 2008, pp. 36–66.

[114] Pickel, B., Schaller, A., Dirigent proteins: Molecular characteristics and potential biotechnological applications. *Appl. Microbiol. Biotechnol.* 2013, *97*, 8427–8438.

[115] Pardo, I., Camarero, S., Laccase engineering by rational and evolutionary design. *Cell Mol Life Sci.* 2015, *72*, 897–910.

[116] Trudeau, D. L., Lee, T. M., Arnold, F. H., Engineered thermostable fungal cellulases exhibit efficient synergistic cellulose hydrolysis at elevated temperatures. *Biotechnol. Bioeng.* 2014, *111*, 2390–2397.

[117] Pei, X. Q., Yi, Z. L., Tang, C. G., Wu, Z. L., Three amino acid changes contribute markedly to the thermostability of β-glucosidase BglC from *Thermobifida fusca*. *Bioresour. Technol.* 2011, *102*, 3337–3342.

[118] Scott, B. R., St-Pierre, P., Lavigne, J. A., Masri, N. et al., *Novel lignin-resistant cellulase enzymes*. WO Patent 2010/096931.

[119] Lavigne, J., Hill, C., Tremblay, A., St-Pierre, P. et al., *Cellulase variants with reduced inhibition by glucose*. WO Patent 2009/089630.

[120] Adney, W. S., Jeoh, T., Beckham, G. T., Chou, Y-C. et al., Probing the role of *N*-linked glycans in the stability and activity

of fungal cellobiohydrolases by mutational analysis. *Cellulose* 2009, *16*, 699–709.

[121] Yi, Z-L., Pi, X-Q., Wu, Z-L., Introduction of glycine and proline residues onto protein surface increases the thermostability of endoglucanase CelA from *Clostridium thermocellum*. *Bioresource Technol.* 2011, *102*, 3636–3638.

[122] Thongekkaew, J., Ikeda, H., Masaki, K., Iefuji, H., Fusion of cellulose binding domain from *Trichoderma reesei* CBHI to *Cryptococcus* sp. S-2 cellulase enhances its binding affinity and its cellulolytic activity to insoluble cellulosic substrates. *Enzym. Microb. Technol.* 2013, *52*, 241–246.

[123] Vu, V. H., Kim, K., Improvement of cellulase activity using error-prone rolling circle amplification and site-directed mutagenesis. *J. Microbiol. Biotechnol.* 2012, *22*, 607–613.

[124] Telke, A. A., Zhuang, N., Ghatge, S. S., Lee, S. H. et al., Engineering of family-5 glycoside hydrolase (Cel5A) from an uncultured bacterium for efficient hydrolysis of cellulosic substrates. *PLoS One* 2013, *8*, e65727.

[125] Lee, H. L., Chang, C-K., Teng, K-H., Liang, P-H., Construction and characterization of different fusion proteins between cellulases and β-glucosidase to improve glucose production and thermostability. *Bioresource Technol.* 2011, *102*, 3973–3976.

[126] Liu, M., Yu, H., Co-production of a whole cellulose system in *Escherichia coli*. *Biochem. Eng. J.* 2012, *69*, 204–210.

[127] Liu, M., Xie, W., Xu, H., Gu, J. et al., Directed evolution of an exoglucanase facilitated by a co-expressed β-glucosidase and construction of a whole engineered cellulase system in *Escherichia coli*. *Biotechnol. Lett.* 2014, *36*, 1801–1807.

[128] Chen, C. C., Luo, H., Han, X., Lv, P. et al., Structural perspectives of an engineered β-1,4-xylanase with enhanced thermostability. *J. Biotechnol.* 2014, *189*, 175–182.

[129] Kazuyo, F., Hong, S. Y., Yeon, Y. J., Joo, J. C. et al., Enhancing the activity of *Bacillus circulans* xylanase by modulating the flexibility of the hinge region. *J. Ind. Microbiol. Biotechnol.* 2014, *41*, 1181–1190.

[130] Bao, X., Huang, X., Lu, X., Li, J. J., Improvement of hydrogen peroxide stability of *Pleurotus eryngii* versatile ligninolytic peroxidase by rational protein engineering. *Enzym. Microb. Technol.* 2014, *54*, 51–58.

[131] Morales, M., Mate, M. J., Romero, A., Martínez, M. J. et al., Two oxidation sites for low redox potential substrates: A directed mutagenesis, kinetic, and crystallographic study on *Pleurotus eryngii* versatile peroxidase. *J. Biol. Chem.* 2012, *287*, 41053–41067.

[132] Mollania, N., Khajeh, K., Ranjbar, B., Hosseinkhani, S., Enhancement of a bacterial laccase thermostability through directed mutagenesis of a surface loop. *Enzym. Microb. Technol.* 2011, *49*, 446–452.

[133] Geisler, D. A., Sampathkumar, A., Mutwil, M., Persson, S., Laying down the bricks: Logistic aspects of cell wall biosynthesis. *Curr. Opin. Plant Biol.* 2008, *11*, 647–652.