The Implications of Lignocellulosic Biomass Chemical Composition for the Production of Advanced Biofuels

NADAV SOREK, TREVOR H. YEATS, HEIDI SZEMENYEI, HEATHER YOUNGS, AND CHRIS R. SOMERVILLE

The majority of terrestrial biomass accumulates as plant cell walls, the main structural component of leaves, stems, roots, fruits, and seeds. The main constituents of plant cell walls are lignin and polysaccharides, which can be transformed into liquid fuel molecules through chemical transformation or microbial fermentation. Because of the large scale of demand for fuel, it is essential that biomass-to-fuel conversion processes maximize conservation of energy in the products. Here, we summarize some of the challenges posed to these processes by the chemical complexity of plant cell walls.

Keywords: biochemistry, biofuels, plant biology, energy sources

The word *biofuel* currently refers primarily to the conversion of sugar or depolymerized starch from food or feed crops to ethanol by fermentation or the conversion of vegetable oil or other sources of lipids to *biodiesel*, a mixture of fatty acid methyl esters. In 2010, approximately 23 billion gallons of ethanol and approximately 6 billion gallons of biodiesel were produced worldwide. However, we think it likely that the use of food or feed crops to produce biofuels will gradually give way to the use of the structural polysaccharides that constitute the bodies of plants. This transition will increase the amount of relevant biomass that can be obtained per hectare, will increase sustainability, and will encourage diversification of the agricultural landscape in other desirable ways (Somerville et al. 2010, Youngs and Somerville 2012).

The development of the suite of technologies required to produce lignocellulosic (LC) fuels at costs that allow direct competition with petroleum-based fuels has been challenging for many reasons. Nevertheless, a number of companies are building the first generation of LC fuel production facilities (box 1). One of the most important obstacles is related to the fact that LC biomass is chemically complex. Therefore, the catalysts used to depolymerize biomass must accelerate many different types of chemical reactions, the chemical separation processes are complex, and the organisms that convert the depolymerized biomass components to fuels must be capable of many different types of metabolic conversion. In addition, the composition of plant biomass differs from species to species and even among cell types within a species (Knox 2008, Popper et al. 2011). In this brief overview, we summarize some of the features of the chemical composition of plant biomass that affect LC biofuel production and outline some of the implications for the development of feedstock crops and the conversion processes used with them.

Overview of the process of LC fuel production

There are currently two main routes to the production of LC fuels: the thermal route and bioconversion. The thermal route depends on the use of high temperatures, in a controlled atmosphere that prevents direct combustion, to decompose the biomass. In the extreme case, the carbon compounds in the biomass can be rapidly converted to *syngas*, a mixture of carbon monoxide, carbon dioxide, hydrogen, and relatively small amounts of other gases. The carbon monoxide and hydrogen can be separated from the other components and then used to synthesize hydrocarbons by the Fischer–Tropsch process, which has been used to make liquid hydrocarbons from coal and natural gas. This method is relatively insensitive to the exact composition of the biomass, although high amounts of inorganic material, such as salts, can be problematic. The process is also capital intensive and is therefore generally considered feasible only at very large scales that would be difficult to support with biomass because of logistical issues such as transportation costs.

Pyrolysis is a thermal decomposition process that takes place at a lower temperature than gasification does and leads to the production of a large number of small fragments

BioScience 64: 192–201. The Author(s) 2014. Published by Oxford University Press on behalf of the American Institute of Biological Sciences.
doi:10.1093/biosci/bit037 Advance Access publication 12 February 2014

Box 1. Commercialization of lignocellulosic biofuels.

As of the writing of this article, there are only two commercial-scale lignocellulosic biofuel facilities in the world. A 20-million-gallon-per-year biorefinery in Crescentino, Italy, owned by Beta Renewables, uses a steam pretreatment and biological conversion process to produce lignocellulosic fuel from a variety of perennial grasses, corn stover, wheat and rice straw, and poplar. A facility owned by Kior in Columbus, Missouri, is expected to produce 13 million gallons per year of gasoline and diesel by pyrolysis of forestry residues. In addition, at least a dozen companies, including Beta Renewables and Kior, are in the midst of building commercial-scale lignocellulosic refineries in the United States that are expected to begin production sometime between the end of 2013 and the fourth quarter of 2015. If all of the plants are completed, the combined capacity would be roughly 230 million gallons per year. Feedstocks for the additional facilities vary dramatically, from dedicated energy crops to residues and municipal solid waste. Several of the companies have stated plans to replicate their technology, either by building more plants themselves or through licensing structures to other companies for implementation. Many experts in the industry expect a lag in production scale up as these first commercial facilities go online and improvements are developed. The International Energy Agency forecasts that biofuels could supply 5%–15% of transportation energy (3.7–8.2 million barrels of oil equivalents per day) by 2035.

of biomass. Although some gas is generated, the majority of the biomass ends up as a liquid called *pyrolysis oil* or a solid called *biochar*. Depending on the conditions, a typical pyrolysis reactor may produce more than 300 compounds, of which dehydrated sugars and organic acids are among the most abundant. The technical challenge is to develop robust methods to convert the major components of pyrolysis oils to stable, nonviscous, noncorrosive fuel molecules. Pyrolysis reactors can be scaled to match the availability of biomass and have moderate capital costs. Several demonstration-scale facilities are currently operating in the United States.

Bioconversion processes generally have four main components: (1) a pretreatment process in which biomass is ground and treated briefly with hot water or steam that may also contain a dilute acid or base to partially depolymerize the biomass; (2) an enzymatic process in which glycan-depolymerizing enzymes (i.e., cellulases, hemicellulases, pectinases, and polysaccharide oxygenases) cleave polysaccharides to sugars; (3) a fermentation process in which sugars are converted to fuel, usually ethanol; and (4) a separation process, usually distillation, in which fuel is separated from water and residual solids. Although ethanol is currently the main product of fermentation-based approaches, we consider it likely that other types of liquid fuels, such as isobutanol, will eventually be produced by fermentation, using genetically modified microorganisms. There is a very large number of possible variants of these component processes. Perhaps the most extreme is the use of a strong mineral acid to completely depolymerize the polysaccharides to sugars. Most of the variations do not convert lignin to liquid fuel; it is usually recovered as a solid that is mechanically dehydrated and burned to produce the heat and power for the bioconversion processes.

The composition of biomass

Cell walls are the major component of plant biomass. The composition of cell walls varies widely among species (Popper et al. 2011) and may vary within an individual, depending on the cell type or in response to environmental conditions (Knox 2008). However, relatively little is known about how or why the amount or composition of cell walls is regulated. In the context of this discussion, the main significance of cell wall variation is that biofuel production processes must be robust to such variation.

Some cell types, such as those that make up the vascular tissues, have very thick walls that are usually rigidified with lignin. These cell types, which can constitute the majority of mass in a plant body, typically undergo a period of cell wall deposition to form "secondary cell walls" after cell expansion is complete. Other cell types, such as the mesophyll cells of leaves have relatively thin cell walls that minimize diffusive resistance to gases and the fluxes of the products from photosynthesis. Therefore, it appears that the primary determinants of the quantity and composition of cell walls are developmental controls (Etchells et al. 2012).

From a biofuels perspective, cell walls can be considered to be composed of five main components: cellulose, hemicellulose, pectin, lignin, and minerals that are collectively referred to as *lignocellulose* (table 1). The following sections describe these components and their relevance in biofuel production.

Cellulose. Cellulose is the major load-bearing component of plant cell walls and is thought to be the most abundant biopolymer on Earth (Somerville 2006). Cellulose microfibrils are insoluble, cable-like structures composed of approximately 24 hydrogen-bonded chains containing $\beta(1,4)$-linked glucose molecules (Guerriero et al. 2010, Fernandes et al. 2011). The glucan chains are parallel, and successive glucose residues are rotated 180 degrees to form a repeating disaccharide unit called *cellobiose*. This allows the glucan chains to form a flat, relatively inflexible, ribbon-like crystalline structure held together by hydrogen bonds and Van der Waals forces to form microfibrils. Hydroxyl groups present in cellulose macromolecules are involved in a number of intra- and intermolecular hydrogen bonds, which result in various ordered crystalline arrangements. Because of the way the chains are stacked to form the crystalline array,

Table 1. The composition of selected lignocellulosic feedstocks.

Source	Composition of biomass (percentage)				Key features	Reference
	Cellulose	Lignin	Hemicellulose	Ash		
Miscanthus giganteus (perennial grass)	37–45	17–21	19–25	1–3	Abundant ferulic and coumaric acid esters	Haffner et al. 2013
Pine (softwood tree)	25–42	18–26	21–30	0.3–2	Hemicellulose rich in mannans, low ash, lignin enriched for guaiacyl units	*www.nrel.gov/biomass*, Sánchez 2009
Poplar (hardwood tree)	4–55	18–25	24–40	1–4	Lignin enriched for syringyl units	*www.nrel.gov/biomass*
Agave tequilana (arid climate succulent)	31–55	7–12	8–17	3–7	The juice of fresh tissue is rich in soluble sugars and pectin	*www.nrel.gov/biomass*

there are several distinct faces to a cellulose microfibril. The degree of polymerization can vary from 500 to 15,000 glucose molecules, depending on the tissue type (Somerville 2006).

Cellulose can be depolymerized into cellobiose and glucose by the combined action of endo- and exoglucanases, glucosidases (Wilson 2009), and polysaccharide monooxygenases (PMOs). It is thought that these enzymes act on only one face of the microfibril. Therefore, depolymerization is necessarily a relatively slow process in which the outermost glucan chains on one face are nicked by endoglucanases or PMOs and then progressively depolymerized by reducing-end and non-reducing-end exoglucanases before the underlying chains can be acted on. Therefore, significantly longer reaction times are needed for cellulose hydrolysis than for the depolymerization of comparable quantities of starch, an α-linked polymer of glucose that is the basis of some food-based biofuels.

It appears that cellulose microfibrils may have amorphous regions in which the crystalline structure has not formed during synthesis or has been disrupted. The definition of *amorphous cellulose* is ambiguous, but it is generally referred to as regions of microfibrils with nonordered β(1,4)-linked glucose molecules that can be detected by changes in X-ray diffraction (Thygesen et al. 2005). Acid treatments can cause a breakdown of the cellulose fibril into roughly 150-nanometer fragments. It is assumed that this is because of the differential acid lability of the crystalline and amorphous regions. Similarly, it is believed that the amorphous regions are more readily attacked by cellulases than are the crystalline regions. However, nothing is known about how amorphous cellulose arises. It is possible that it is an artifact of the methods used to prepare cell walls for analysis. If such regions exist naturally, it is possible to imagine that dispersing the amorphous regions more evenly across the cellulose fibril would enhance cellulose degradation by both acid and enzyme-mediated treatments.

Plant genomes harbor many endogenous genes for cellulases (e.g., the *Arabidopsis* and rice genomes contain 25 and 21 putative genes for cellulases, respectively), but their role is unknown. It seems likely that most plant cellulases are involved in some step of plant development; therefore, the regulation of the expression will be crucial. Several patents describe the production of transgenic plants in which cellulases have been placed under inducible expression. The inventors claim that such constructs may decrease the crystallinity properties of cellulose in plants when they are induced, without affecting the plant's morphology (Klose et al. 2013).

Cellulose fibrils are generally coated with *hemicellulose*, which is hydrogen bonded to the microfibril surface (figure 1). Because the hemicellulose is usually branched, it may help to prevent the aggregation of cellulose microfibrils. Indeed, paper is made by stripping hemicellulose and lignin from cellulose, with the result that the cellulose fibrils hydrogen bond to each other. In support of this idea, it was shown that a mutant of *Arabidopsis* deficient in xyloglucan had highly aggregated cellulose fibrils (Anderson et al. 2010). It would be interesting to test whether plants overproducing xyloglucan might have thinner cellulose fibrils that can be depolymerized more readily.

Recently, it was suggested that a small fraction of xyloglucan is commonly entrapped in the cellulose microfibrils (Wang et al. 2012). These insertions may be what is perceived as amorphous cellulose and may serve as initiation sites for cellulose degradation. Common biomass pretreatments, such as dilute acid, hydrolyze the majority of the hemicellulose and, therefore, increase the fraction of amorphous cellulose close to these tight interactions. This also leads to a lower degree of polymerization of the cellulose. A better understanding of the xyloglucan–cellulose interaction may allow the manipulation of cell wall biosynthesis to enhance the occurrence of these insertions.

Except for the possible existence of amorphous regions, cellulose fibrils are monotonous structures, so there are not many ways to envision that genetic variations or engineered modifications of the cellulose synthetic machinery could improve the suitability of cellulose for making biofuels. However, an analysis of the enzymatic depolymerization of cellulose from several mutants of *Arabidopsis* carrying point mutations in cellulose synthase subunits indicated that these mutants have a type of cellulose that is more readily depolymerized than that of the wild type (Harris et al. 2012). It was hypothesized that the mutations may alter the process of glucan crystallization, which may lead to the formation of microfibrils with a less-crystalline form of cellulose. The

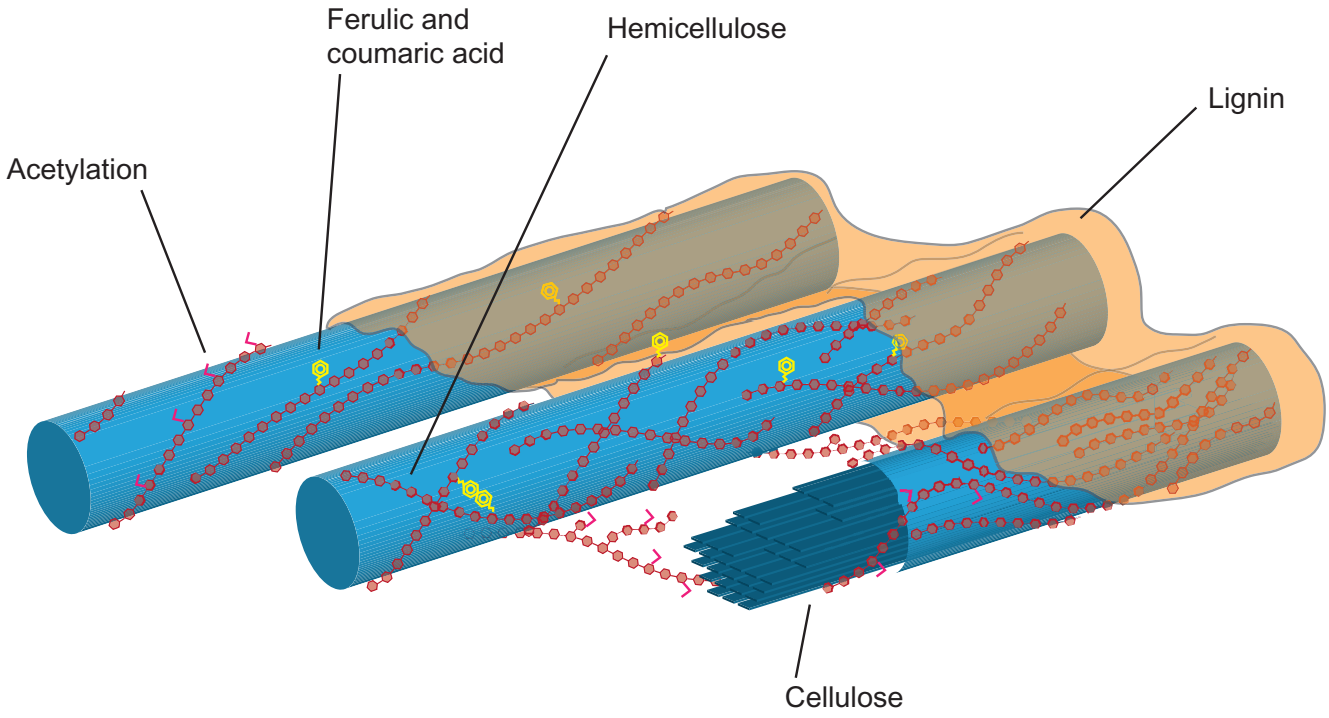

Figure 1. The major structural components of plant cell walls (not to scale).

expression of a mutant cellulose synthase gene in transgenic tobacco resulted in a large increase in cellulose digestibility (Sahoo et al. 2013). Therefore, it might ultimately be possible to develop modified plants with more-digestible cellulose.

Hemicellulose. *Hemicellulose* is a general designation for cell wall polysaccharides that are neither cellulose nor pectin. Most hemicelluloses have a β(1,4)-linked glucan, xylan, galactan, mannan, or glucomannan backbone that is branched with single or longer glycosyl residues (Pauly et al. 2013). In addition, some species have mixed-linkage β(1-4):β(1-3) glucans. These are abundant in some grass species, including major cereals. Secondary cell wall hemicelluloses make up roughly half of the carbohydrates and one-third of the total biomass in the woody tissues and stems considered for biofuel production. Although hemicelluloses represent a potentially large fermentable pool of sugar, they also present challenges to biofuel production. Hemicelluloses are a diverse class of linear and branched polysaccharides that differ widely in composition among plant tissues and species and that change markedly during plant development. They function as load-bearing, cross-linking agents in the wall, binding cellulose bundles, lignins, cell wall proteins, pectins, and nonstructural polysaccharides through a variety of covalent and noncovalent interactions. The monosaccharide constituents of hemicellulose vary widely in their suitability as carbon sources for different forms of microbial fermentations, and the linkages that constitute the various structures of hemicellulose can affect the efficiency of the hydrolysis of the polymers. Hemicelluloses can be roughly divided into four major classes according to their backbone structures.

Xylans are the most abundant hemicellulose component in grasses such as switchgrass (*Panicum virgatum*) and *Miscanthus* and angiosperm hardwoods, such as eucalyptus, willow, and aspen (*Populus* spp.). Xylans are composed of a linear backbone of β(1-4)-linked xylosyl units, which is most commonly decorated with arabinosyl, xylosyl, or glucuronic acid substituents (Scheller and Ulvskov 2010). The arabinosyl side chains may be further linked to glucuronic acid, which is frequently modified by *O*-methyl and *O*-acetyl groups. Other possible side-chain modifications include the phenolic compounds ferulic acid and *p*-coumaric acid, which can form covalent cross-links with each other or with the lignin polymer (Scheller and Ulvskov 2010). All of these modifications alter the solubility of the hemicellulose polymer in water and other polar solvents and affect its hydrogen bonding to cellulose fibrils in the wall. The amount of xylan and the pattern of substitution are highly variable among different species. The secondary cell walls of hardwoods, such as birch and aspen, typically contain 20%–30% 4-*O*-methylglucuronoxylans, whereas arabinoglucuronoxylans are present as the major hemicellulose in grasses (40%–50%) and are a minor component (5%–15%) in softwoods, such as spruce and pine.

Mannans are the major hemicellulose component in some gymnosperm softwood species, although they are also present as a minor component in some hardwoods. Mannans consist of a β(1-4)-linked backbone of mannosyl units, whereas glucomannans contain varying amounts of

randomly interspersed glucosyl units that can constitute up to 50% of the linear backbone (Scheller and Ulvskov 2010, Pauly et al. 2013). This backbone is often decorated by galactosyl side chains and *O*-acetyl groups, particularly in some softwood species (Verhertbruggen et al. 2011).

Galactans are branched soluble polymers principally composed of β(1-3) or β(1-4) galactosyl units that are normally minor components of secondary cell walls (Laine 2005, Caffall and Mohnen 2009). The β(1-4) galactans, which are most commonly observed, are considered hemicelluloses because the planar structure associated with the β(1-4) linkage facilitates hydrogen bonding to the surface of cellulose microfibrils. The heartwood of larch contains 10%–15% β(1-3) galactan substituted with arabinose (Laine 2005). Because *β*(1-3) galactans would not be expected to hydrogen bond to the surface of cellulose fibrils, they may play a role different from that of *β*(1-4) galactans. When woody tissues of gymnosperms are subjected to mechanical forces such as bending, the compressed tissue (compression wood) contains 7%–12% acidic galactan that consists of a *β*(1-4) galactosyl backbone substituted with galacturonic acid residues at about every 20 residues (Laine 2005). In contrast, the tension wood that develops opposite to the compression site in angiosperms contains a complex *β*(1-4) galactan polymer with galactan, glucuronic acid, and rhamnogalactan branching (Teleman 2009).

Noncellulosic glucans are also a minor component of biomass, because they are typically associated with primary cell walls and early stages of growth and development. Occurring as a homopolymer of *β*(1-4)-linked glucosyl units or as a mixed-linkage polymer of *β*(1-4) glucan with interspersed *β*(1-3) glucosyl linkages in a variety of patterns, they vary among plant species and tissues. The latter polymer, termed *mixed-linkage glucan*, is prevalent in grasses but has also been found in other species (Popper et al. 2011). Xyloglucan, a *β*(1-4) glucan with a variety of branch patterns and substituents (Scheller and Ulvskov 2010), is a hemicellulose that is prevalent in vegetative tissues but may also be present in very minor amounts in stems and woody tissues. Glucans are water soluble, amenable to acid and enzyme degradation, and easily fermentable, which makes them desirable components for biofuel production. Mutants such as maize *candy leaf-1*, which accumulate mixed-linkage glucans in mature tissue and exhibit increased saccharification yields, could be interesting models for modification of other biofuel feedstocks (Pauly et al. 2011).

The ratio of hemicellulose polymer types and their structures not only vary from species to species and tissue to tissue, but they are also modified during growth, development, and maturation, which causes heterogeneity issues for biomass processing. For example, there is an increase in glucomannans and a decrease in glucuronoarabinoxylan in the transition from early- to latewood in pine (Saka 2001). Although the effects of the full complement of hemicellulose structures on their association with cellulose and lignin remain poorly understood, there are clear effects on biomass properties. For example, glucomannans appear to have better adsorption to cellulose than xylans do (Clayton and Phelps 1965), and the sorption is reduced by both acetylation and the presence of galactosyl side chains (Hannuksela et al. 2002). These effects have clear ramifications for pretreatment strategies and for thoughtful engineering of biomass with altered wall components.

Hemicelluloses respond differently to biomass pretreatments. The glycosidic linkages in hemicelluloses are relatively labile to acid hydrolysis, which allows complete depolymerization by relatively mild acid pretreatment. However, the liberated pentose sugars, such as xylose and arabinose, are particularly susceptible to dehydration under dilute acidic conditions at high temperatures and form toxic furaldehydes. In addition, not all organisms used in commercial fermentation can use pentose sugars, especially in the presence of glucose. Ferulic, coumaric, uronic, and acetic acids are also released during acid hydrolysis. The release of these acids from the biomass by hot-water treatments can allow them to act as a sort of endogenous catalyst, performing autohydrolysis. In contrast, dilute alkaline conditions solubilize hemicellulose oligomers and hydrolyze ester linkages with a minor effect on glycosidic linkages. Because basic pretreatments do not completely saccharify hemicelluloses, additional enzymes may be required in downstream processing. The combination of complex linkage types in hemicelluloses is particularly challenging for enzymatic digestion. For example, a typical grass xylan could require up to seven enzymes for efficient depolymerization: endoxylanase, exoxylanase, *β*-xylosidase, arabinosidase, glucuronidase, acetyl xylan esterase, and feruloyl esterase. Although engineering plants with simplified hemicellulose may be attractive, the impact on wall integrity and compensatory changes in wall structure are not well understood.

Although several hemicellulose-deficient mutants and several genes involved in hemicellulose synthesis have been identified (Scheller and Ulvskov 2010), few have been sufficiently tested with the biochemical resolution needed to implement rational biomass engineering. For example, it was recently shown that in *xax1*, a rice mutant deficient in the xylosyl modification of arabinose residues on arabinoxylan, the mutant plants exhibit an increased extractability of xylan and increased saccharification, presumably because of decreased cross-linking to lignin (Chiniquy et al. 2012).

Some mutations affecting cell wall polysaccharide composition have negative effects on plant growth, development, and resiliency, especially in terms of vascular tissue integrity. For example, glucuronyl transferase genes (*GUX*) in the GT8 family have been identified in *Arabidopsis* (Mortimer et al. 2010). In *gux* mutants, which lack both glucuronic acid and 4-*O*-methyl-glucuronic acid modifications of xylan, the unbranched xylan was easier to extract under alkaline conditions, most likely because of decreased covalent linkages to lignin. Although the plants did not show decreases in growth under controlled conditions, they did have weakened stems; therefore, they could be prone to lodging under field

conditions or could be susceptible to xylem collapse under water stress.

Pectin. *Pectins* are heterogeneous, water-soluble polymers that fill in the cellulose–hemicellulose matrix in primary cell walls and accumulate in the middle lamella between cells, providing adhesion. With a high content of methylated and acidic sugars—usually galacturonic acid—some pectins are highly branched and structurally very complex (Caffall and Mohnen 2009). These polymers can chelate calcium and form gels. In secondary cell walls, the pectin fraction is negligible and is therefore not considered an important source of sugar in advanced biofuel production. However, pectins are abundant in waste residues, such as sugar beet pulp, citrus waste, apple pomace, and potato pulp, which could be used as feedstocks for biofuel production. One of the major candidates for feedstock production in arid lands is *Agave* species, which are also rich in pectins. The use of pectins for biofuel production was recently reviewed by Xiao and Anderson (2013).

Lignin. *Lignin* is an amorphous, irregular polymer of phenylpropanoid monomers in the cell walls of higher plants that might be considered a natural plastic. During plant growth, monomer units are secreted into the forming cell wall, where they infiltrate the polysaccharide matrix and are polymerized by a free-radical process that leads to a randomized structure. The result is a rigid protective barrier around the polysaccharides that allows the formation of specialized tissues for water transport, supports vertical growth, and prevents pathogen invasion.

Lignin interferes with biomass saccharification in nature and in biofuel production by occluding and protecting cellulose fibers from depolymerization. Lignin also binds and inactivates cellulolytic enzymes and can produce degradation products that inhibit fermentation. As a result, most biofuel conversion pathways have a pretreatment step to modify, reduce, or remove lignin and increase saccharification, which adds cost and increases energy requirements. In general, the use of pretreatment and saccharification efficiency are influenced by four factors: (1) total lignin content, (2) lignin composition, (3) lignin degree of polymerization, and (4) the extent and nature of lignin–polysaccharide cross-linking. The latter features are affected both by the lignin and carbohydrate composition and by the conditions in the cell wall during polymerization.

Plant species differ both in the total amount of lignin and in the structure of lignin they contain, which affects the efficiency of different pretreatments. In general, softwoods from gymnosperms such as pine contain more lignin (25%–35% of the cell wall) than do hardwoods such as poplar (18%–25%) or grasses (10%–30%) (Sánchez 2009). The pattern of methoxyl substitutions in the lignin precursors of these plants and that within plant tissues also differ. For example, softwood lignins generally contain more guaiacyl units, whereas hardwood lignins have more syringyl units, and grass lignins contain both, along with higher amounts of hydroxybenzyl units (Ziebell et al. 2010). Some lignins may contain other modifications formed through the preferential enzymatic acylation of syringyl units. For example, lignin in grasses can contain up to 40% *p*-coumarate, whereas 80% of lignins in agave can be acetylated, and lignins in palm, poplar, and willow contain relatively high amounts of *p*-hydroxybenzoate (Withers et al. 2012).

The composition of lignin monomers affects polymerization and cross-linking patterns in the wall. For example, syringyl units, which have methoxy substituents on C3 and C5 positions of the phenyl ring, are described as being less condensed (Cesarino et al. 2012). They form fewer recalcitrant carbon–carbon bonds and tend to form more linear chains but have more β–β resin structures. In contrast, guaiacyl units, which contain a single methoxy substituent on C3 of the phenyl ring and therefore have an additional site for cross-linking, are more branched and have been implicated in restricting fiber swelling under some pretreatment conditions (Ramos et al. 1992). Finally, hydroxyphenyl units contain no methoxy substituents. These units are usually a minor component—less than 5% of lignin—and appear to be important in the early stages of lignification. The lack of methoxy substituents allows more carbon–carbon bonds but also shifts the stability of the radicals formed during oxidation such that fewer β-*O*-4′ aryl ethers are formed, which results in polymers with a lower molecular weight (Ziebell et al. 2010).

The bonds in lignin are very difficult to break under normal conditions. This is why woody tissues can persist in the environment for years. Lignin can be removed from plant cell walls to varying degrees by extreme treatments including heat, acids, bases, oxidization agents, and solvents. Most pretreatments cleave ester and ether linkages to varying degrees. During lignification, several types of covalent bonds are formed between the lignin monomers, including β aryl ethers, esters, and carbon–carbon bonds. The most common intramolecular linkages in lignin are β-*O*-aryl ethers formed by coupling the 4-hydroxyl group of the phenyl ring with the propanoid side chain of another subunit. β-*O*-4′ aryl ethers represent 30%–50% of linkages in wood and up to 90% of linkages in grasses, and many pretreatments, including dilute acid, preferentially cleave these linkages (Villaverde et al. 2009). In contrast, ester linkages, which can be high in some grasses, are broken under alkaline conditions. Carbon–carbon linkages in lignin are the most recalcitrant, but they can be cleaved by oxidative catalysts.

The fact that the ratio of syringyl to guaiacyl units (the S:G ratio) clearly affects saccharification efficiency in some cases highlights the complexity of lignification and cell wall chemophysical structure. Syringyl units are the preferred target for acylation (Withers et al. 2012), and they clearly also play a confounding role in the patterns of cross-linking within lignin polymers and in forming lignin–polysaccharide linkages. For example, lignin monomers acetylated at the end of the propanoid side chain cannot form resinols, form

fewer β–β carbon bonds, and appear to favor β aryl ether formation, which results in shorter, more linear lignins, which, in turn, enhances pretreatment efficiency (del Río et al. 2008). However, coumarylation may favor more extensive cross-linking to carbohydrates. The relevance of the S:G ratio to saccharification may depend on the total lignin content (Studer et al. 2011).

Cross-linking between lignins and the side chains of branched polysaccharides such as pectins and hemicelluloses may have the most influence on saccharification. These are of two types: ethers and esters. *p*-coumarylated polymers may form more carbohydrate-benzyl ethers than other monomers. Coumarylated and ferulolyated polymers can both form esters. Unlike carbohydrate-benzyl ethers, lignin–lignin and lignin–polysaccharide esters are easily cleaved during alkaline pretreatments but not with dilute acid. Alkaline-cleavable esters can also form between oxidized rings and sugars containing uronic acid substituents (Takahashi and Koshijima 1988).

The genetic manipulation of lignin content and composition to improve saccharification has been reviewed in several recent papers (e.g., Vanholme et al. 2012a). Most research has been focused on enzymes of the phenylpropanoid biosynthetic pathways. Although the results have been mixed, trends are emerging. Although many of the genes can be downregulated to reduce lignin content and improve saccharification, this often has unwanted effects on growth and pathogen resistance. The manipulation of transcriptional control of lignification and the engineering of novel lignin subunits and linkages have recently emerged as more-precise ways to improve biomass feedstocks for specific conversion routes.

Engineering lignins to contain more labile linkages, such as esters, has been proposed to enhance saccharification with alkaline pretreatment and to reduce processing temperatures. For example, increasing feruloylated lignin (Grabber et al. 2008) and introducing *O*-diphenolic precursors, such as 4-hydroxyconiferyl alcohol, rosmarinic acid (Tobimatsu et al. 2012), dicaffeoyl quinate, and epigallocatechin gallate (Elumalai et al. 2012) have been shown to enhance the removal of lignin after pretreatment. These units participate in normal β-*O*-4′ aryl ether formation but may suppress lignin-carbohydrate cross-linking. Methods of reducing the degree of polymerization of lignin include overexpressing a modified methyl transferase to produce 4-*O*-methyl lignin precursors that do not form β-*O*-4′ aryl ethers (Zhang et al. 2012) and expressing a bacterial lyase gene that cleaves the phenyl group of phenylpropanoids (Eudes et al. 2012). Both strategies successfully enhance saccharification without affecting plant growth. The emerging knowledge of transcription factors that govern cell wall composition and lignification in specific tissues, coupled with new knowledge of feedback effects of changes in lignins will allow more creative and directed alterations in cell wall or biomass properties (Vanholme et al. 2012b).

Ash and silica. In some plant species, a significant fraction of the total biomass is not combustible and is recovered as ash from bioenergy processes. The amounts range from about 2% of dry weight in *Miscanthus* to about 8% in canary grass (Baker and Elbersen 2005). The amount of ash and its composition can affect LC bioprocessing. For example, in conversion systems that depend on combustion of residual solids following polysaccharide depolymerization, the ash ends up in the boilers that are used for producing heat and power and can amount to as much as 20% of solids (Baker and Elbersen 2005). Some types of ash, such as those high in potassium, are highly corrosive to the boilers. In addition, at combustion temperatures, silica may react with potassium or calcium to form alkali silicates of low melting point that can foul the equipment with slag (Monti et al. 2008). Whatever the case, ash must be removed from the boiler and disposed of, which increases operating costs. Woody species generally have much lower ash content than do herbaceous species, which leads to wood being the preferred feedstock for biomass conversion processes that are particularly sensitive to ash (e.g., pyrolysis, gasification, dissolution in strong acid; Baxter 1993).

The five most abundant elements in ash are silicon, potassium, calcium, sulfur, and chlorine (Baker and Elbersen 2005). Silica is frequently the most abundant component of ash and seems most amenable to reduction by breeding or genetic modification of the feedstock, because it does not seem to have an indispensible role. This would be particularly useful in the case of rice, which has so much silica in the straw that the material is generally not considered suitable for use as a biofuel feedstock. The recent discovery that silica and arsenic are transported by the same transporters (Ma et al. 2008) and the desire to reduce arsenic content in rice grain may lead to rapid progress in the development of low-silica varieties of rice. Opportunities to modify the other major components of ash are less obvious. The fact that the mineral content of plants varies significantly, depending on the growth conditions, suggests that there may be significant scope to optimize mineral content through agronomic practices or through breeding and selection. The mineral content of leaves may also vary widely from that of stems (Monti et al. 2008), which suggests that harvest practices may be used to manage the ash content in the biorefinery.

Acetylation of cell wall polysaccharides. Aside from cellulose, most cell wall polysaccharides are *O*-acetylated to some extent. The presence of *O*-acetylation can inhibit enzymatic saccharification (Selig et al. 2009), and acetate present in crude hydrolysates can inhibit growth and fermentation by yeast and other microorganisms (Palmqvist and Hahn-Hägerdal 2000). This suggests that reducing cell wall acetylation may be a promising approach for improving LC feedstocks.

The molecular mechanism of polysaccharide *O*-acetylation in plants has only recently begun to be understood. In

Arabidopsis, a family of four REDUCED WALL ACETYLATION (*RWA*) genes was identified on the basis of their similarity to the Cas1p protein that is required for polysaccharide *O*-acetylation in yeast (*Cryptococcus neoformans*). A loss-of-function mutation (*rwa2*) was associated with an approximately 15% reduction in the amount of cell wall acetylation in seedling leaves. These reductions were attributed to a reduction in the acetylation of both pectin and xyloglucan (Manabe et al. 2011). Mutation of all four *RWA* genes indicated that they are also involved in xylan acetylation in the secondary cell wall of the stem. However, the loss of function in all four *RWA* genes resulted in an only 40% reduction of nonpectin acetylation, which indicates that there are additional pathways for cell wall acetylation (Lee et al. 2011).

Although it appears that *rwa* mutants are reduced in acetylation across structural classes of polysaccharides, the acetylation of specific classes of polysaccharides may depend on proteins belonging to the *TRICHOME BIREFRINGENCE-LIKE* (*TBL*) family. The *TBL27* gene *Altered Xyloglucan 4* (*AXY4*) is required for the *O*-acetylation of xyloglucan (Gille et al. 2011), whereas mutation of *TBL29*/*ESK1* appears to primarily affect the *O*-acetylation of xylan (Xiong et al. 2013). Fourier transform infrared spectroscopy analysis of other *tbl* mutants has indicated decreased cell wall ester functionality that has not been resolved to specific polymers, although the composition and quantity of pectin is altered in both *pmr5* (Vogel et al. 2004) and *tbr* (Bischoff et al. 2010) mutants, which suggests that these genes may affect the acetylation of pectin.

The function of cell wall acetylation is not known, although the pleiotropic effects of lesions in this pathway indicate that some types of acetylation of cell walls are not dispensable to normal plant growth. Although *axy4* shows no visible phenotype, *pmr5* is dwarf and resistant to powdery mildew (Vogel et al. 2004), *tbl29*/*esk1* exhibits collapsed xylem and enhanced cold tolerance (Xiong et al. 2013), and the quadruple *rwa1/2/3/4* mutant exhibited decreased stem mechanical strength (Lee et al. 2011). This suggests that modifications of acetylation must be carefully targeted in order to avoid undesired side effects. One possibility is that acetylation could be replaced with an alternative *O*-linked derivative that could impart similar hydrophobicity or steric bulk as the acetyl group. Some cell wall polysaccharides are modified with *O*-methyl ethers, and understanding the mechanism of *O*-methylation might provide a means of reengineering cell wall polysaccharides that are otherwise acetylated. To this end, a glucuronoxylan methyltransferase (GXMT1) required for the methylation of glucuronic acid side chains of 4-*O*-methyl glucuronoxylan was recently identified in *Arabidopsis* (Urbanowicz et al. 2012). If the substrate specificity of such enzymes could be modified to mirror that of essential polysaccharide acetyltransferases, it may be possible to engineer biomass that does not release acetate during pretreatment or hydrolysis.

Nonstructural polysaccharides. All higher plants produce starch as an energy storage polymer. Leaf starch normally accumulates during the day and is degraded at night, such that it is almost depleted at dawn. The *CORNGRASS1* gene of maize is a microRNA gene that promotes juvenile cell wall identity and leads to leaf starch accumulation by controlling several transcription factors. Ectopic expression of the *CORNGRASS1* gene in *Arabidopsis*, *Brachypodium*, maize, and switchgrass resulted in plants with higher starch levels in their vegetative tissues (Chuck et al. 2011). Because of the ease of starch depolymerization, these plants exhibited increased sugar yields in digestibility assays. This is an interesting proof of concept for the idea that it might be possible to engineer plants with improved utility for liquid fuel production because of the accumulation of large amounts of nonstructural polysaccharides during senescence. Some plants store fructans, which might have similar utility for biofuel applications.

The physiological reason that plants expend energy to accumulate starch rather than storing carbon in soluble sugars is that starch is less osmotic than a corresponding quantity of free glucose molecules. However, starch granules occupy space and may drive up the concentration of other molecules by volume exclusion. Therefore, when tobacco plants were modified to overproduce starch granules, the turgor pressure increased by 41%, and the osmotic pressure was increased by 126% (Hoffmann-Benning et al. 1997), and a similar effect was also shown in potatoes. Plants use turgor pressure as the driving force for cell expansion, and cell growth and polarity are based on the plastic response of the wall to the mechanical force exerted by the turgor pressure. Since changes in turgor can have negative effects on growth and development, it is important to understand the mechanistic basis for altered turgor in the starch-modified plants.

Several species store energy in the form of cell wall polysaccharides. *Nasturtium* (*Tropaeolum majus*); the tropical tree *Copaifera langsdorffii*; *Hymenaea courbaril*; and the most common example, tamarind (*Tamarindus indica*) use xyloglucan for energy in cotyledons (dos Santos et al. 2004). The proportion and arrangement of the oligosaccharide side chains in xyloglucans can give rise to chains with conformational differences, which could display different properties (Hayashi 1989). On this basis, it is worth considering the modification of bioenergy crops to store energy as cell wall polysaccharides instead of as starch.

Cotyledon cell walls of *Nasturtium* seeds are thickened with xyloglucan, a single seed weighing about 120 milligrams that contains about 30 milligrams of xyloglucan. After germination, the xyloglucan disappears and the cotyledon cell walls thin markedly. Except for the absence of terminal fucosyl units linked to the branching galactosyl residues, the seed reserve xyloglucan and the structural xyloglucan from primary cell walls of dicotyledonous tissues are very similar (Fry 1989). The four main enzymes responsible for xyloglucan degradation in *Nasturtium* have been isolated and show

specificity for the storage xyloglucan structure so that other components of the tissue are not affected (Tiné et al. 2000).

Conclusions

The chemical complexity of plant cell walls poses many challenges in the effort to produce LC fuels. As has been summarized here, there are potential opportunities to genetically modify the composition of cell walls of biofuel feedstocks to facilitate the production of LC fuels and bioproducts. Some of the factors that facilitate the enzymatic depolymerization of cell walls for biofuel production are also likely to improve the digestibility of plant biomass by ruminants. However, there are major deficiencies in our knowledge of how the polymers that constitute cell walls are synthesized and deposited. Very little is known about the fine structure of cell walls or why the composition varies from one cell type to another, one species to another, or within species in response to environmental conditions. Although the progress in understanding plant cell walls has accelerated in recent years with the advent of improved analytical techniques and the proliferation of genome sequences, there are major unresolved fundamental questions about the logic of cell wall composition. Therefore, in some respects, understanding plant cell wall structure and function is one of the last poorly explored frontiers in basic biology. The fact that the favorite model organisms of the biomedical sciences (e.g., yeast, fungi, bacteria, animals) do not have structurally related cell walls means that, unlike for many other aspects of plant biology, comparative studies with nonplants are generally of limited utility in this respect.

Because cell walls have important functional roles in growth and development and in biotic interactions, it seems likely that a lot of tuning may be required to find the optimal balance between biological function and the optimal composition for the production of biofuels or forage. In this respect, it may be useful to bear in mind that, although the end of the petroleum era is many decades away, the eventual transition to renewable fuels and chemical feedstocks will ultimately be necessary. Therefore, even if it takes many years of research to understand how to optimize biomass composition for the production of fuels and chemicals, it is a topic worth exploring.

Acknowledgments

All of the authors receive a salary from a grant from the energy company BP to the University of California, Berkeley. CRS owns shares of Mendel Biotechnology and LS9, two companies with business interests related to advanced biofuels. NS was the recipient of Postdoctoral Award no. FI-434-2010 from the Binational Agricultural Research and Development Fund. This work was funded in part by the Energy Bioscience Institute.

References cited

Anderson CT, Carroll A, Akhmetova L, Somerville C. 2010. Real-time imaging of cellulose reorientation during cell wall expansion in *Arabidopsis* roots. Plant Physiology 152: 787–796.

Baker RR, Elbersen HW. 2005. Managing ash content and quality in herbaceous biomass: An analysis from plant to product. Paper presented at the 14th European Biomass Conference; 17–21 October 2005, Paris, France.

Baxter LL. 1993. Ash deposition during biomass and coal combustion: A mechanistic approach. Biomass and Bioenergy 4: 85–102.

Bischoff V, Nita S, Neumetzler L, Schindelasch D, Urbain A, Eshed R, Persson S, Delmer D, Scheible WR. 2010. TRICHOME BIREFRINGENCE and its homolog AT5G01360 encode plant-specific DUF231 proteins required for cellulose biosynthesis in *Arabidopsis*. Plant Physiology 153: 590–602.

Caffall KH, Mohnen D. 2009. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydrate Research 344: 1879–1900.

Cesarino I, Araújo P, Sampaio Mayer JL, Paes Leme AF, Mazzafera P. 2012. Enzymatic activity and proteomic profile of class III peroxidases during sugarcane stem development. Plant Physiology and Biochemistry 55: 66–76.

Chiniquy D, et al. 2012. XAX1 from glycosyltransferase family 61 mediates xylosyltransfer to rice xylan. Proceedings of the National Academy of Sciences 109: 17117–17122.

Chuck GS, et al. 2011. Overexpression of the maize Corngrass1 microRNA prevents flowering, improves digestibility, and increases starch content of switchgrass. Proceedings of the National Academy of Sciences 108: 17550–17555.

Clayton DW, Phelps GR. 1965. Sorption of glucomannan and xylan on alpha-cellulose wood fibers. Journal of Polymer Science C 11: 197–220.

Del Río JC, Rencoret J, Marques G, Gutiérrez A, Ibarra D, Santos JI, Jiménez-Barbero J, Zhang L, Martínez ÁT. 2008. Highly acylated (acetylated and/or *p*-coumaroylated) native lignins from diverse herbaceous plants. Journal of Agricultural and Food Chemistry 56: 9525–9534.

Dos Santos HP, Purgatto E, Mercier H, Buckeridge MS. 2004. The control of storage xyloglucan mobilization in cotyledons of *Hymenaea courbaril.* Plant Physiology 135: 287–299.

Elumalai S, Tobimatsu Y, Grabber JH, Pan X, Ralph J. 2012. Epigallocatechin gallate incorporation into lignin enhances the alkaline delignification and enzymatic saccharification of cell walls. Biotechnology for Biofuels 5 (art. 59).

Etchells JP, Moore L, Jiang WZ, Prescott H, Capper R, Saunders NJ, Bhatt AM, Dickinson HG. 2012. A role for *BELLRINGER* in cell wall development is supported by loss-of-function phenotypes. BMC Plant Biology 12 (art. 212).

Eudes A, et al. 2012. Biosynthesis and incorporation of side-chain-truncated lignin monomers to reduce lignin polymerization and enhance saccharification. Plant Biotechnology Journal 10: 609–620.

Fernandes AN, Thomas LH, Altaner CM, Callow P, Forsyth VT, Apperley DC, Kennedy CJ, Jarvis MC. 2011. Nanostructure of cellulose microfibrils in spruce wood. Proceedings of the National Academy of Sciences 108: E1195–E1203.

Fry SC. 1989. The structure and functions of xyloglucan. Journal of Experimental Botany 40: 1–11.

Gille S, de Souza A, Xiong GY, Benz M, Cheng K, Schultink A, Reca IB, Pauly M. 2011. *O*-acetylation of *Arabidopsis* hemicellulose xyloglucan requires AXY4 or AXY4L, proteins with a TBL and DUF231 domain. Plant Cell 23: 4041–4053.

Grabber JH, Hatfield RD, Lu F, Ralph J. 2008. Coniferyl ferulate incorporation into lignin enhances the alkaline delignification and enzymatic degradation of cell walls. Biomacromolecules 9: 2510–2516.

Guerriero G, Fugelstad J, Bulone V. 2010. What do we really know about cellulose biosynthesis in higher plants? Journal of Integrative Plant Biology 52: 161–175.

Haffner FB, Mitchell VD, Arundale RA, Bauer S. 2013. Compositional analysis of *Miscanthus giganteus* by near infrared spectroscopy. Cellulose 20: 1629–1637. doi:10.1007/s10570-013-9935-1

Hannuksela T, Tenkanen M, Holmbom B. 2002. Sorption of dissolved galactoglucomannans and galactomannans to bleached kraft pulp. Cellulose 9: 251–261.

Harris DM, et al. 2012. Cellulose microfibril crystallinity is reduced by mutating C-terminal transmembrane region residues CESA1A903V and CESA3T942I of cellulose synthase. Proceedings of the National Academy of Sciences 109: 4098–4103.

Hayashi T. 1989. Xyloglucans in the primary cell wall. Annual Review of Plant Physiology 40: 139–168.

Hoffmann-Benning S, Willmitzer L, Fisahn J. 1997. Analysis of growth, composition and thickness of the cell walls of transgenic tobacco plants expressing a yeast-derived invertase. Protoplasma 200: 146–153.

Klose H, Günl M, Usadel B, Fischer R, Commandeur U. 2013. Ethanol inducible expression of a mesophilic cellulase avoids adverse effects on plant development. Biotechnology for Biofuels 6 (art. 53).

Knox JP. 2008. Revealing the structural and functional diversity of plant cell walls. Current Opinion in Plant Biology 11: 308–313.

Laine C. 2005. Structures of Hemicelluloses and Pectins in Wood and Pulp. PhD dissertation. Helsinki University of Technology, Helsinki, Finland.

Lee C, Teng Q, Zhong R, Ye ZH. 2011. The four *Arabidopsis* REDUCED WALL ACETYLATION genes are expressed in secondary wall-containing cells and required for the acetylation of xylan. Plant and Cell Physiology 52: 1289–1301.

Ma JF, Yamaji N, Mitani N, Xu X-Y, Su Y-H, McGrath SP, Zhao F-J. 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proceedings of the National Academy of Sciences 105: 9931–9935.

Manabe Y, et al. 2011. Loss-of-function mutation of REDUCED WALL ACETYLATION2 in *Arabidopsis* leads to reduced cell wall acetylation and increased resistance to *Botrytis cinerea*. Plant Physiology 155: 1068–1078.

Monti A, Di Virgilio N, Venturi G. 2008. Mineral composition and ash content of six major energy crops. Biomass and Bioenergy 32: 216–223.

Mortimer JC, et al. 2010. Absence of branches from xylan in *Arabidopsis gux* mutants reveals potential for simplification of lignocellulosic biomass. Proceedings of the National Academy of Sciences 107: 17409–17414.

Palmqvist E, Hahn-Hägerdal B. 2000. Fermentation of lignocellulosic hydrolysates II: Inhibitors and mechanisms of inhibition. Bioresource Technology 74: 25–33.

Pauly M, Hake S, Kraemer FJ. 2011. Maize variety and method of production. Patent no. US20110302669 A1. US Patent and Trademark Office.

Pauly M, Gille S, Liu L, Mansoori N, de Souza A, Schultink A, Xiong G. 2013. Hemicellulose biosynthesis. Planta 238: 627–642.

Popper ZA, Michel G, Hervé C, Domozych DS, Willats WGT, Tuohy MG, Kloareg B, Stengel DB. 2011. Evolution and diversity of plant cell walls: From algae to flowering plants. Annual Review of Plant Biology 62: 567–590.

Ramos LP, Beruil C, Saddler JN. 1992. Comparison of steam pretreatment of eucalyptus, aspen, and spruce wood chips and their enzymatic hydrolysis. Applied Biochemistry and Biotechnology 34/35: 37–48.

Sahoo DK, Stork J, DeBolt S, Maiti IB. 2013. Manipulating cellulose biosynthesis by expression of mutant *Arabidopsis proM24::CESA3$^{ixr1\text{-}2}$* gene in transgenic tobacco. Plant Biotechnology Journal 11: 362–372.

Saka S. 2001. Chemical composition and distribution. Pages 59–88 in Hon DN-S, Shiraishi N, eds. Wood and Cellulosic Chemistry, 2nd ed. revised. CRC Press.

Sánchez C. 2009. Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnology Advances 27: 185–194.

Scheller HV, Ulvskov P. 2010. Hemicelluloses. Annual Review of Plant Biology 61: 263–289.

Selig MJ, Adney WS, Himmel ME, Decker SR. 2009. The impact of cell wall acetylation on corn stover hydrolysis by cellulolytic and xylanolytic enzymes. Cellulose 16: 711–722.

Somerville C. 2006. Cellulose synthesis in higher plants. Annual Review of Cell and Developmental Biology 22: 53–78.

Somerville C, Youngs H, Taylor C, Davis SC, Long SP. 2010. Feedstocks for lignocellulosic biofuels. Science 329: 790–792.

Studer MH, DeMartini JD, Davis MF, Sykes RW, Davison B, Keller M, Tuskan GA, Wyman CE. 2011. Lignin content in natural Populus variants affects sugar release. Proceedings of the National Academy of Sciences 108: 6300–6305.

Takahashi N, Koshijima T. 1988. Ester linkages between lignin and glucuronoxylan in a lignin-carbohydrate complex from beech (*Fagus crenata*) wood. Wood Science and Technology 22: 231–241.

Teleman A. 2009. Hemicelluloses and pectins. Pages 101–110 in Ek M, Gellerstedt G, Henriksson G, eds. Wood Chemistry and Wood Biotechnology. DeGruyter.

Thygesen A, Oddershede J, Lilholt H, Thomsen AB, Ståhl K. 2005. On the determination of crystallinity and cellulose content in plant fibres. Cellulose 12: 563–576.

Tiné MAS, Cortelazzo AL, Buckeridge MS. 2000. Xyloglucan mobilisation in cotyledons of developing plantlets of *Hymenaea courbaril* L. (Leguminosae-Caesalpinoideae). Plant Science 154: 117–126.

Tobimatsu Y, Elumalai S, Grabber JH, Davidson CL, Pan X, Ralph J. 2012. Hydroxycinnamate conjugates as potential monolignol replacements: *In vitro* lignification and cell wall studies with rosmarinic acid. ChemSusChem 5: 676–686.

Urbanowicz BR, et al. 2012. 4-*O*-methylation of glucuronic acid in *Arabidopsis* glucuronoxylan is catalyzed by a domain of unknown function family 579 protein. Proceedings of the National Academy of Sciences 109: 14253–14258.

Vanholme R, Morreel K, Darrah C, Oyarce P, Grabber JH, Ralph J, Boerjan W. 2012a. Metabolic engineering of novel lignin in biomass crops. New Phytologist 196: 978–1000.

Vanholme R, Storme V, Vanholme B, Sundin L, Christensen JH, Goeminne G, Halpin C, Rohde A, Morreel K, Boerjan W. 2012b. A systems biology view of responses to lignin biosynthesis perturbations in *Arabidopsis*. Plant Cell 24: 3506–3529.

Verhertbruggen Y, Yin L, Oikawa A, Scheller HV. 2011. Mannan synthase activity in the CSLD family. Plant Signaling and Behavior 6: 1620–1623.

Villaverde JJ, Li J, Ek M, Ligero P, de Vega A. 2009. Native lignin structure of *Miscanthus x giganteus* and its changes during acetic and formic acid fractionation. Journal of Agricultural and Food Chemistry 57: 6262–6270.

Vogel JP, Raab TK, Somerville CR, Somerville SC. 2004. Mutations in PMR5 result in powdery mildew resistance and altered cell wall composition. Plant Journal 40: 968–978.

Wang T, Zabotina O, Hong M. 2012. Pectin-cellulose interactions in the *Arabidopsis* primary cell wall from two-dimensional magic-angle-spinning solid-state nuclear magnetic resonance. Biochemistry 51: 9846–9856.

Wilson DB. 2009. Cellulases and biofuels. Current Opinion in Biotechnology 20: 295–299.

Withers S, Lu F, Kim H, Zhu Y, Ralph J, Wilkerson CG. 2012. Identification of grass-specific enzyme that acylates monolignols with *p*-coumarate. Journal of Biological Chemistry 287: 8347–8355.

Xiao C, Anderson CT. 2013. Roles of pectin in biomass yield and processing for biofuels. Frontiers in Plant Science 4: 67.

Xiong G, Cheng K, Pauly M. 2013. Xylan *O*-acetylation impacts xylem development and enzymatic recalcitrance as indicated by the *Arabidopsis* mutant *tbl29*. Molecular Plant. doi:10.1093/mp/sst014

Youngs H, Somerville C. 2012. Growing better biofuel crops. Scientist 26: 46–52.

Zhang K, Bhuiya M-W, Pazo JR, Miao Y, Kim H, Ralph J, Liu C-J. 2012. An engineered monolignol 4-*O*-methyltransferase depresses lignin biosynthesis and confers novel metabolic capability in *Arabidopsis*. Plant Cell 24: 3135–3152.

Ziebell A, Gracom K, Katahira R, Chen F, Pu Y, Ragauskas A, Dixon RA, Davis M. 2010. Increase in 4-coumaryl alcohol units during lignification in alfalfa (*Medicago sativa*) alters the extractability and molecular weight of lignin. Journal of Biological Chemistry 285: 38961–38968.

Nadav Sorek is a postdoctoral fellow, Trevor Yeats is a postdoctoral fellow, Heidi Szemenyei is a research associate, Heather Youngs is a senior analysis fellow, and Chris R. Somerville (crs@berkeley.edu) is a professor at the University of California, Berkeley.