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MINI-REVIEW

Challenges of the utilization of wood polymers: how can they be overcome?

Yunqiao Pu · Matyas Kosa · Udaya C. Kalluri · Gerald A. Tuskan · Arthur J. Ragauskas

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Abstract Diminishing fossil fuel resources as well as growing environmental and energy security concerns, in parallel with growing demands on raw materials and energy, have intensified global efforts to utilize wood biopolymers as a renewable resource to produce biofuels and biomaterials. Wood is one of the most abundant biopolymer composites on earth that can be converted into biofuels as well as used as a platform to produce bio-based materials. The major biopolymers in wood are cellulose, hemicelluloses, and lignin which account for >90% of dry weight. These polymers are generally associated with each other in wood cell walls resulting in an intricate and dynamic cell wall structure. This mini-review provides an overview of major wood biopolymers, their structure, and recent developments in their utilization to develop biofuels. Advances in genetic modifications to overcome the recalcitrance of woody biomass for biofuels are discussed and point to a promising future.

Keywords Wood biopolymer · Biofuels · Genetic modification · Biodiesel

Y. Pu
Institute of Paper Science and Technology,
Georgia Institute of Technology,
Atlanta, GA, USA

Y. Pu · U. C. Kalluri · G. A. Tuskan (✉) · A. J. Ragauskas (✉)
BioEnergy Science Center,
Oak Ridge, TN, USA
e-mail: Tuskanga@ornl.gov

A. J. Ragauskas
e-mail: arthur.ragauskas@chemistry.gatech.edu

M. Kosa · A. J. Ragauskas
School of Chemistry and Biochemistry,
Georgia Institute of Technology,
Atlanta, GA, USA

U. C. Kalluri · G. A. Tuskan
Oak Ridge National Laboratory,
Oak Ridge, TN, USA

Introduction

Growth in the global population and affluence translates into rapidly rising raw material consumption and energy demand worldwide. These demands coupled with diminishing fossil fuel resources and ever-increasing environmental concerns have spurred global efforts to develop alternative sustainable forms of fuels from renewable sources including woody biomass (Ragauskas et al. 2006a; Pu et al. 2008). Our global forest resources represent one of the most abundant biopolymer sources on earth with a wood production capacity of approximately 3,900 million m^3/year (http://www.fao.org/forestry/energy/en/).

Wood has always contributed to mankind's development and is an integral part of modern society. In the future, its role will only grow as it will play a critical role in carbon capture and is a fundamental feedstock for bio-based fuels, chemicals, materials, and power. Currently, the greatest processing challenge is to develop efficient deconstruction and separation technologies that enable the release of sugar and aromatic compounds 'locked in' the intricacy of wood cell wall macromolecular structures as summarized in Fig. 1.

In the biorefinery concept, wood derived sugars and lignin can be converted to fuels, chemicals, and value-added materials using biological and thermochemical technologies, while process residues are used for energy generation (Ragauskas et al. 2006a; Foust et al. 2008;

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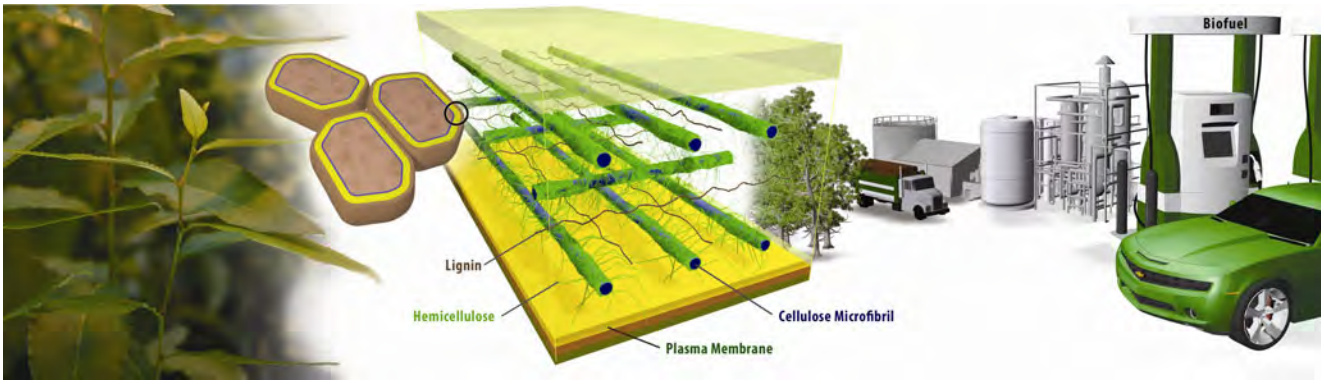


Fig. 1 Illustration of plant cell wall and biofuel opportunities

Sannigrahi et al. 2010). This mini-review highlights recent developments in the conversion of woody biomass to biofuels and genetic modification of wood which will reduce its recalcitrance and simplify the conversion of wood polymers to renewable fuels.

Structure of wood biopolymers

Wood is a natural three-dimensional biocomposite composed of cellulose, hemicelluloses, and lignin (>90%), with minor amounts of inorganics and extractives. Wood generally is classified into two groups: coniferous wood or softwood, obtained from gymnosperm trees, and hardwood, obtained from angiosperm trees. The major biopolymers of wood cell walls are sugar-based polymers (i.e., cellulose and hemicellulose, 65–75%) and lignin (18–35%; Rowell et al. 2000). On a dry-weight basis, wood typically has an elemental composition of about 49% carbon, 6% hydrogen, and 44% oxygen (Haygreen and Bowyer 1996). The cell types and chemical compositions vary between hardwood and softwood as well as among species. In general, softwood species have a comparable cellulose content (40–44%), higher lignin (26–34%), and lower hemicellulose content (20–32%) as compared to hardwood species (cellulose 40–44%, lignin 23–30%, hemicellulose 15–35%; Haygreen and Bowyer 1996; Rowell et al. 2000).

The cell wall in wood is a complicated and dynamic structure, consisting of three anatomical regions: the middle lamella, the primary wall, and the secondary wall. The latter unit, contributing up to ~89% of the mass of mature trees, is made up three layers: the outer (S1), the middle (S2), and the inner (S3) layers (Rowell et al. 2000). Cell walls are typically deposited by layers upon synthesis, with the primary cell wall formed during cell growth as the first wall laid down in dividing and growing plant cells, and the secondary cell wall deposited when cell growth has ceased (Mohnen et al. 2008). The middle lamella is the layer between two neighboring cells. The thickness of each layer and its constituents vary in different cell types, tissues, and wood species. The ultrastructure of cell wall is generally considered to be comprised of long cellulose microfibrils held together by hemicellulose and lignins. Recently developed analytical methods, such as atomic force microscopy (AFM) and single molecule methods, have advanced our knowledge about the ultrastructure of plant cell walls. For example, using advanced AFM techniques, Ding and Himmel (2006) proposed a model of plant cell wall cellulose elementary fibril that consists of 36 β-1,4-glucan chain, with hemicellulose particles interacting with the surface of the chain to form a ribbon-like bundle of microfibril. Key challenges that must be addressed include the need to obtain higher spatial resolution of the locations of the biopolymers, their topochemical function, and the relationship between lignin, hemicelluloses, and cellulose deposition and how this contributes to biomass recalcitrance.

The ease with which wood polymers are converted to value-added fuels, chemicals, and materials depends upon our practical knowledge of their structure, as well as our ability to manipulate the chemistry and biochemistry of cellulose, hemicellulose, and lignin. Wood cellulose is a linear homopolymer of (1→4)-β-D-glucopyranosyl units with a varying degree of polymerization (DP) (Fig. 2; Hallac and Ragauskas, 2011). The cellulose chain has a strong tendency to form intramolecular and intermolecular hydrogen bonding by the hydroxyl groups on these linear glucan chains, which stiffens the chains and promotes cellulose aggregations. Aggregated cellulose has characterized to be crystalline cellulose using X-ray and two crystal phases (I_α and I_β), which were detected using solid-state nuclear magnetic resonance (NMR) in 1980s (Atalla and VanderHart 1984). An averaging degree of crystallinity of ~50–70% was observed in wood cellulose using solid-state NMR (Ragauskas et al. 2006b). Although crystallinity has been widely studied in wood cellulose ultrastructure during the last three decades, recent evidence has suggested that the microfibrils/nanofibrils of cellulose in higher plants

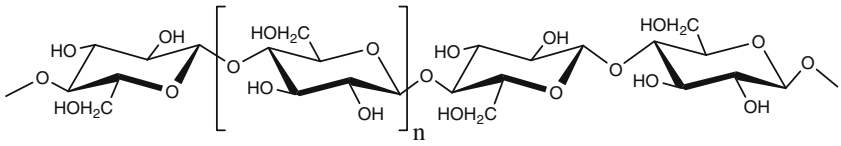
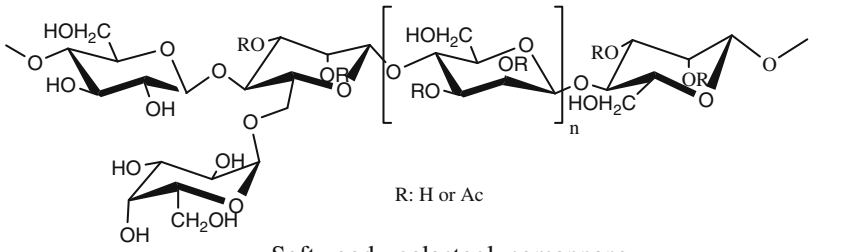


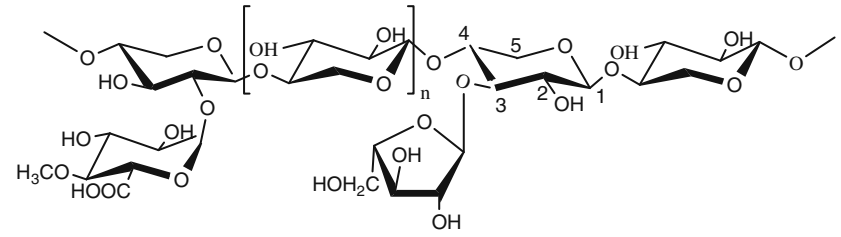
Fig. 2 Molecular structure of cellulose (n:DP)

including wood poses a helical twist in their native state, which is not consistent with the assumptions of the symmetry of the crystal structures under crystallographic analysis. Atalla et al. (2008) therefore proposed to use the term "aggregate" to indicate the highly ordered cellulose fibrils. Although it is well known that reduced cellulose crystallinity can facilitate enzymatic deconstruction, it remains yet to be determined to what extent this fundamental property can be genetically altered in native systems to reduce recalcitrance. A recent report by Foston et al. (2009) demonstrated significant variation in cellulose crystallinity for a series of wild poplar samples from ~54% to 68%, but the extent to which this was due to genetic and/or environmental variability was not determined.

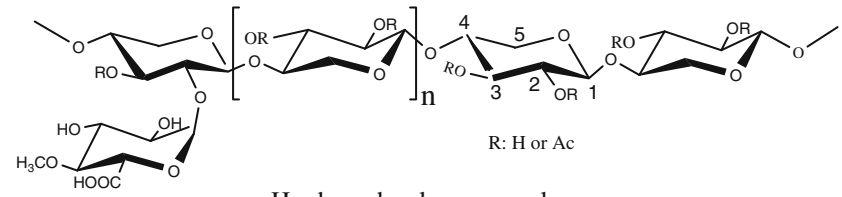
After cellulose, the next major polysaccharide resource in wood is hemicelluloses. Unlike cellulose, hemicelluloses have a much lower DP (i.e., generally 50–300), frequently have side chain groups and are essentially amorphous (Pu et al. 2008; Harris and Stone 2008). The major hemicelluloses in softwoods are galactoglucomannans and arabinoglucuronoxylan, while in hardwood, the predominant hemicelluloses are glucuronoxylan (Fig. 3). Hardwood glucuronoxylan and softwood arabinoglucuronoxylan both have a backbone of (1–4)-linked β-D-xylopyranosyl units, but exhibit differences in branching and substitution patterns. In hardwoods, the xylan polymer is usually acetylated at C_2- and C_3-positions (i.e., ~3.5–7.0 acetyl groups/10 xylose) and is also branched with small amounts of (1→2)-linked pyranoid 4-*O*-methyl-α-D-glucuronic acid units. For softwoods, the xylan polymer is not acetylated, but branched with (1→2)-linked pyranoid 4-*O*-methyl-α-D-glucuronic acid and (1→3)-linked α-L-arabinofuranosyl units, with a typical arabinose:uronic acid:xylose ratio of ~1:2:8. Galactoglucomannan is comprised of (1→4)-linked β-D-glucopyranosyl and D-mannopyranosyl units that are partially acetylated at the C_2- and C_3-positions. Galactoglucomannan is more important than arabinoglucuronoxylan



Softwood - galactoglucomannans



Softwood - arabinoglucuronoxylan



Hardwood - glucuronoxylan

Fig. 3 Principal structures of wood hemicelluloses

in softwoods, contributing 15–20% of the dry wood mass. In addition, sugar components in hemicellulose can take part in the formation of lignin–carbohydrate complexes (LCCs) by covalent linkages between lignin and carbohydrates. Despite significant analytical efforts directed at characterizing LCCs, they remain poorly defined and their biosynthetic pathways need further investigation.

Recently research efforts using two-dimensional NMR $^{13}C–^{1}H$ correlation techniques such as HMBC/HMQC have identified the ester/ether linkages of LCC (Balakshin et al. 2003, 2007). For example, in the lignin–carbohydrate ester linkages in loblolly pine, an uronic acid residue was observed to attach to the γ-position of lignin side chain, (Balakshin et al. 2007).

Of the three major biopolymers that constitute wood, lignin is chemically different from the other macromolecular polymers. This biopolymer is an amorphous polyphenolic polymer that is synthesized by enzymatic dehydrogenative polymerization of 4-hydroxyphenyl propanoid units. The biosynthesis of lignin is generally considered to stem from the polymerization of three types of phenylpropane units as monolignols: coniferyl, sinapyl, and *p*-coumaryl alcohol (Fig. 4; Davin and Lewis 2005; Boerjan et al. 2003). Softwood lignin is composed mainly of coniferyl alcohol units and minor amounts of *p*-hydroxyphenyl units, while hardwood lignin is generally composed of coniferyl and sinapyl alcohol units with minor amounts of *p*-hydroxyphenyl units. Although the structure of lignin is usually regarded as irregular and highly heterogeneous with no regular extended repeating unit structures observed, recent studies on biosynthesis of lignin by altering the expression of individual genes of phenylpropanoid and monolignol biosynthetic pathways have suggested a structure of lignin that has a predominantly linear lignin chain (Vanholme et al. 2008; Stewart et al. 2009). While the exact structure of protolignin is unknown, the most common interunit linkages in softwood/hardwood lignin have been identified (Fig. 5) with the improvements in methods for identifying lignin degradation products and advancements in spectroscopic methods (Chakar and Ragauskas 2004; Pu et al. 2008). Many of these advances have been spurned by the use of advanced NMR techniques supported by lignin model compound synthesis. For example, in the early 1990s the

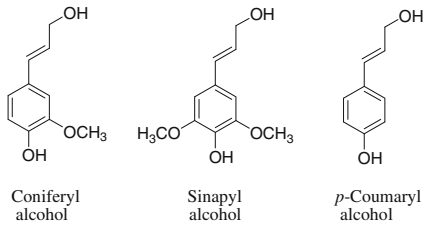


Fig. 4 Phenyl propanoid monolignol units in the biosynthesis of softwood lignin

detection of dibenzodioxocin in softwoods at a level of ~10–15% initiated a flurry of research efforts that has led to several new sub-unit structures to be detected (Karhunen et al. 1995; Brunow et al. 1998; Zhang and Gellerstedt 2001; Kukkola et al. 2004; Zhang et al. 2006).

Conversion of wood biopolymers to biofuels with emphasis on bioethanol

Utilizing softwood and hardwood forest resources for biofuel production can be separated into two fundamentally different approaches: thermal and biological conversions (Ragauskas et al. 2006a). The aim of both routes is fuels that can be used for as a replacement for gasoline and/or diesel. In light of the elemental composition of lignin, cellulose and hemicelluloses a key conversion issue is efficient depolymerization and deoxgenation of these biopolymers which yields a liquid fuel with a lower oxygen content resulting in higher heating values, hence, better fuel quality properties (Pu et al. 2008).

Thermochemical conversions, such as gasification and pyrolysis, address this issue by applying heat and pressure to varying degrees. Gasification applies significantly higher temperatures and produces a syngas (i.e., CO and H_2) that can be catalytically converted into an array of fuels including methanol, dimethyl ether, ethanol, and short chain alkanes. Different catalysts facilitate this conversion, and the desired end products can be more or less controlled by the process parameters. Key challenges for the gasification of woody resources to liquid fuels include syngas cleanup and conditioning, and the development of fuel synthesis catalysts (Zinoviev et al. 2010). On the other hand, pyrolysis generates a wide array of products, possibly in the hundreds; containing aromatic compounds (e.g., catechols, phenols, etc.) as well as low molecular weight acids, ethers, and alcohols. The latter route requires less capital investment than gasification and is attracting the interest of researchers and industry worldwide (Demirbas 2009). Pyrolysis reactors frequently use an inert atmosphere and yield approximately +50% bio-oil along with a char with reactor temperatures varying typically from 400 to 600°C. The latter product is frequently viewed as a thermal resource for pyrolysis and as a possible resource for biochar and land reclamation efforts. Bio-oils traditionally suffer from high viscosity values and oxygen content, lower heating values, corrosiveness and a tendency to polymerize and age during storage. Practical fuel applications of pyrolysis oils necessitate that these unfavorable properties be removed prior to their utilization as a green diesel or gasoline equivalent. Recent research advances are pursing catalytic systems that either improve the products from pyrolysis and/or can

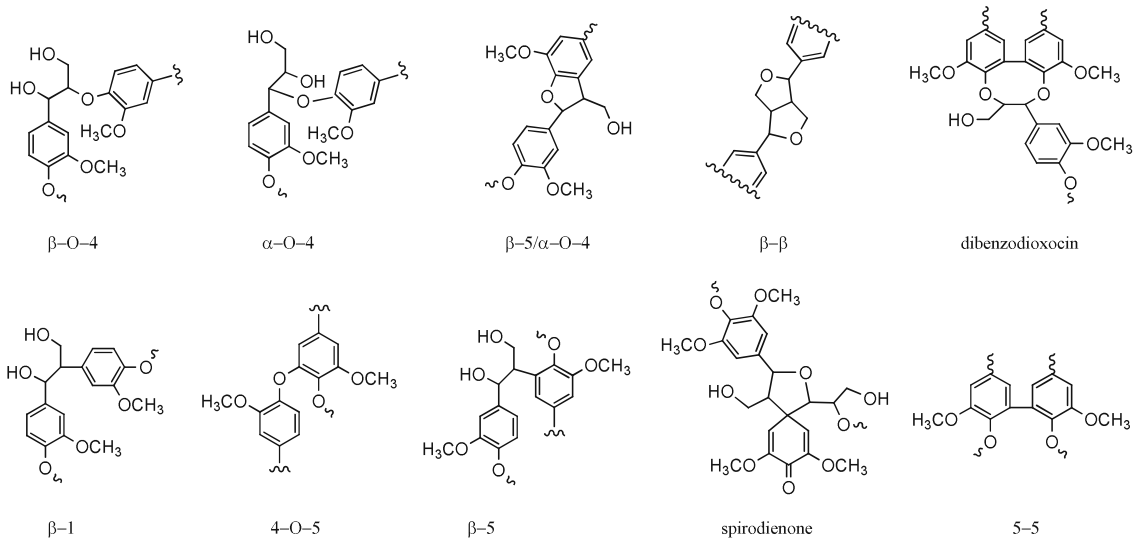


Fig. 5 Primary inter-linkages in softwood lignins

be used downstream for refining bio-oils (Demirbas 2009; Vispute et al. 2010).

The biological route for converting woody biomass to biofuels is contingent on the efficient conversion of plant polysaccharides to monosaccharides, which are subsequently fermented to simple alcohols like ethanol. In terms of theoretical yields, the biological route has a lower theoretical biofuel yield than the gasification route, but practical bio-conversion operations can yield higher yields and favorable co-products. Nonetheless, both pathways provide technical challenges and benefits that need to be tailored to specific regional operational considerations (Singhania et al. 2010, Stephen et al. 2010).

The natural recalcitrance of biomass including wood currently necessitates the use of a pretreatment prior to enzymatic deconstruction of cellulose and hemicelluloses. Dependent on the pretreatment parameters, several properties of biomass are altered including the resulting biomass constituents, cellulose crystallinity, lignin/hemicellulose structure, LLCs, degree of polymerization, fiber size, and pore size. Each of these changes impacts the recalcitrance of pretreated biomass and need to be optimized. A key need in this field is the development of low recalcitrance trees that would minimize or eliminate the need for costly pretreatment technologies. Advances in plant genetics, our fundamental understanding of how plant cell wall topochemistry controls recalcitrance and the growing awareness that even wild trees have significant variations in recalcitrance suggest that future advances in plant engineering will address this challenge in the near future (Johnson and Elander 2008, Studer et al. 2011).

The key operational factors that need to be optimized for an effective pretreatment are good digestibility of polysaccharides; low sugar degradation; minimal generation of toxic by-products; fermentation compatibility; and cost-optimizing considerations including reasonable chip size, low operating temperatures/pressures, minimal use of chemical additives as well as efficient lignin recovery (Alvira et al. 2010). As to be expected, these factors change according to pretreatment applied and substrate utilized. Four pretreatment techniques, i.e., dilute acid, steam explosion, organosolv, and sulfite pretreatment to overcome recalcitrance of lignocellulose have been identified as promising for several wood species (Zhu and Pan 2010; Zhu et al. 2010).

Although traditionally, pretreated woody biomass stream is sequentially enzymatically hydrolyzed and then fermented, the application of simultaneous saccharification and fermentation (SSF) has largely displaced this approach due to savings in capital costs and higher ethanol production efficiency (Söderström et al. 2005). The main drawback of SSF is that there has to be a compromise between the optimal reaction conditions for cellulase and the microorganism applied. Although the crystal structure of multiple cellulases are known, as well as the mechanism for the splitting of cellobioses from the separated cellulose chain, the mechanism of separating individual chains of cellulose from cellulosics fibers remains a field of active research (Rabinovich et al. 2002). Recent research suggests that enzymes can attack at irregularities where the hydrogen bonding between the glucose units is already disrupted. The enzyme's cellulose binding subunit attaches at this site and a crevice in the protein provides lower energy levels for the individual cellulose chain by using increasingly stabilizing noncovalent bonding ("peeling" of an individual chain). Then in the cellulose degrading subunit, the cellulose chain is cut into cellobioses, and the energy released is used to pull another link into the active site (Nimlos et al. 2007). An interesting property of these enzymes is that they are

extracellular: hence, they do not use intracellular energy source to hydrolyze cellulose (Nimlos et al. 2007). Endoenzymes can cut along the cellulose, while exoenzymes only attack at chain ends; additional β-glycosidases are also needed for cellobiose hydrolysis (Rabinovich et al. 2002).

Yeasts like *Saccharomyces cerevisiae* are traditionally used to ferment glucose into ethanol; however, they have limitations involving final alcohol concentrations, temperature, and furfural resistance, although novel strains are constantly being selected that have better tolerance (Söderström et al. 2005; Alper et al. 2006; Rudolf et al. 2008; Hou 2010; Watanabe et al. 2010). Furthermore, the lack of polysaccharide (cellulose and hemicellulose) and pentose to alcohol conversion enzymes gives the greatest challenge in this area (Grange et al. 2010). As a family, hardwoods and softwoods contain broad spectrum of hemicelluloses like glucomannan, galactoglucomannans, arabinoglucuronoxylan, and glucuronoxylans that can release xylose, arabinose, mannose, and galactose during pretreatment, which is valuable if converted into ethanol. Consequently, ongoing research is focusing on identifying new strains to overcome above disadvantages (Rudolf et al. 2008; Matsushika et al. 2009; Grange et al. 2010, Table 1). Modified yeast species, such as *Pichia stipitis* and *Kluyveromyces marxianus* (Rudolf et al. 2008; Zhang et al. 2010, Table 1), and bacteria, such as *Zymomonas mobilis* (Grange et al. 2010; Santos et al. 2010, Table 1), are being applied. Studies with genetically modified bacteria like *Escherichia coli* are also increasingly being pursued (Huerta-Beristain et al. 2008; Grange et al. 2010, Table 1) as well as fermentation condition optimization by experimental design (Santos et al. 2010). Against all of the above disadvantages, yeast (*S. cerevisiae*) is still considered to be the most robust and widely studied strain for fermentation. There are recent successful genetic techniques, such as global transcription machinery engineering (Alper et al. 2006) and genome shuffling (Hou 2010), that increase yields and volumetric productivities while they also increase product tolerance (Table 1).

Recently, consolidated bioprocessing (CBP) has emerged as the most promising process that enables one host species to produce their own cellulases and hemicellulases and also ferment both hexoses (glucose, fructose, galactose, mannose) and pentoses (xylose, arabinose) into ethanol with high efficiency. It is noteworthy that CBP significantly lowers production cost due to in situ enzyme production compared to SSF. CBP can be approached from two fundamental directions: (1) modifying naturally occurring cellulose degrading and sugar fermenting strains to reach better yields; or (2) heterologously expressing cellulases in noncellulolytic strains. Earlier methods utilized anaerobic cellulolytic bacteria (e.g., *Clostridium thermocellum*), while latter methods utilize *E. coli*, *Z. mobilis*, and *S. cerevisiae* as hosts for cellulases from *Trichoderma* and *Aspergillus* fungi. CBP proved more efficient than separate hydrolysis of cellulose partly due to an enzyme–microbe synergy. This synergy is present when cellulose–enzyme–microbe (CEM) complexes can form instead of only cellulose–enzyme (CE) complexes and CEMs hydrolysis efficiency can be several folds higher (Lynd et al. 2005). Cellobiohydrolases (CBH) from multiple sources have already been cloned and expressed in *S. cerevisiae* successfully, with specific activities comparable to native hosts. However, when recombinant *S. cerevisiae* has been grown on cellulose (as sole carbon source) the amount of secreted CBH was reported to be lower than in the native host (Den Haan et al. 2007a, b).

Genetic modifications of wood to reduce recalcitrance

Many of today's challenges of utilizing wood for biofuels via the biological pathways are centered on the very recalcitrant nature of its ultrastructure and chemical constituents. Given the advances in genetics of plant science, it is only natural that researchers would use these tools to enhance the biological conversion properties of woody biomass to biofuels. Nonetheless, the genetic modification of wood, a heterogeneously composed highly variable complex tissue, inherently involves many genes and gene families (Keegstra 2010; Sykes et al. 2008; Dinus et al. 2001). Forward as well as reverse genetics approaches have been employed to identify genes involved in these

Table 1 Several efficient bioethanol producing species

Species	Ethanol yield (g/g substrate)	Productivity (g/l h)	References
Saccharomyces cerevisiae (wild type)	0.35	1.20	Alper et al. 2006
S. cerevisiae spt 15–300	0.40	2.03	
S. cerevisiae S3–10	0.42	2.43	Hou 2010
S. cerevisiae	Np	3.30	Grange et al. 2010
Kluyveromyces marxianus	0.43	1.81	
Escherichia coli	0.57	2.50	
Klebsiella oxytoca	0.52	2.10	
Zymomonas mobilis	0.69	2.29	Santos et al. 2010

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processes (Pilate et al. 2002; Ralph et al. 2006). The extent of our knowledge of mechanisms that characterize wood composition is found to vary according to cell wall component, cell type, and environmental backdrop (Mellerowicz and Sundberg 2008; Kalluri and Keller 2010). For example, the enzymatic steps involved in lignin biosynthesis are fairly well characterized and have been the subject of numerous manipulation experiments (van Parijs et al. 2010); in contrast, the molecular players in cellulose, hemicellulose, and pectin biosynthesis pathways are relatively less annotated. The interplay between and possible coregulation of lignin, cellulose, and hemicellulose biosynthesis is generally not understood. Furthermore, the mechanisms channeling matrix precursors, coordinating membrane transport and roles of structural and regulatory proteins, are just beginning to be scrutinized.

The most characterized subcomponent of the cell wall is lignin (Boerjan et al 2003; Li and Chapple 2010; Vanholme et al. 2010). Lignin is found primarily in the secondary cell wall and plays a role in pathogen resistance, water relations, wound response, and conferring strength for integrity of cell structure as well as the upright habit of plants (Barakat et al 2010; Kitin et al 2010; Tronchet et al. 2010). Monolignol biosynthesis is controlled by roughly ten enzymes (i.e., PAL, C4H, 4CL, C3H, HCT, CCoAOMT, CCR, F5H, COMT, and CAD) leading to the formation of guaiacyl (G), syringyl (S), and hydroxyphenyl (H) subunits of lignin (Voelker et al. 2010). RNAi–mediated down-regulation of C3H expression in hybrid poplar was reported to decrease the lignin content and cause a shift in the monomer composition (Coleman et al. 2008). Downregulation of CCR was shown to decrease lignin levels by nearly 50% (Leplé et al. 2007). Although field-grown hybrid poplar downregulation lines of 4CL were reported to have lower lignin levels and S:G ratios, the same group reported no substantial changes in sacchrification potential (Voelker et al. 2010). This study suggests that recalcitrance of biomass may not have a simplistic correlation with lignin levels in woody plants as has previously been reported for nonwoody plants such as alfalfa (Chen and Dixon 2007). These reports show that shifts in S:G ratio (Ralph et al. 2006), reductions and increases in total lignin (Mansfield 2009), and novel forms of lignin (Stewart et al. 2009) are feasible via genetic modification of individual enzymatic steps in the terminal pathway. Several transcription factors belonging to NAC, MYB, and WRKY gene families have recently been shown to regulate lignin biosynthetic pathway at the plant level (Yamaguchi et al. 2008; Zhou et al. 2009; Guillaumie et al. 2010; Zhong et al. 2010). However, our knowledge of the cell-specific regulation and variation in lignin content and structure across different cell types, developmental stages, and environments and in their interplay with other cell wall components is still at its infancy.

Relatively less known is about the genetic control of cellulose biosynthesis, although a model has been proposed where plasma membrane-integrated rosette complexes and associated ancillary membrane proteins synthesize cellulose microfibrils (Somerville 2006; Joshi and Mansfield 2007; Guerriero et al. 2010). The six-lobed rosette structures have been shown to be composed of the cellulose catalytic subunit, cellulose synthase (CesA). Each of the estimated 36 CesAs within a complex synthesizes individual glucan chains that come together to create a fully formed cellulose microfibril. Certain isoforms of sucrose synthase (SuSy), invertase, sucrose phosphate synthase (SPS), and UDP-glucose pyrophosphorlyase (UGPase) have been shown to be involved in substrate provision to the pathway and the CesA complexes themselves (Haigler et al. 2007; Coleman et al. 2009). However, the roles of other peripheral proteins, such as KORRIGAN (KOR; Nicol et al. 1998), a membrane-bound β-1,4-endoglucanase; COBRA (COB), a glycosyl-phosphatidylinositol (GPI)-anchored protein (Schindelman et al. 2001); KOBITO, a plant-specific membrane-associated protein (Pagant et al. 2002); the chitinase-like CTL1/POM1/ELP1/HOT2 (Zhong et al. 2002; Kwon et al. 2007); and CesA-interactive protein 1 (CSI1), identified in a two-hybrid screen with CesA (Gu et al. 2010), have not been clarified. Recent reports have shown that overexpression of poplar *KOR* gene in *Arabidopsis* increased the levels and decreased the crystallinity of cellulose (Takahashi et al. 2009). While overexpression of *UGPase* resulted in an increase in stress response and a decrease in growth accompanying some increase in cellulose quantity (Coleman et al. 2007), over-expression of *SuSy* in hybrid poplar resulted in clear increased cellulose quantity and crystallinity resulting in thicker walls and increased wood density (Coleman et al. 2009). There, however, are no reports of transcription factors involved in regulating the cellulose biosynthesis pathway (Zhong et al. 2010).

Hemicelluloses in wood including xyloglucan, xylan, mannan, and glucomannans are common in secondary walls (Harholt et al. 2010; Scheller and Ulvskov 2010). The amounts as well as length and side-chain substitution pattern of xylans, specifically glucuronoxylan and arabinoxylan, have a contributing role in recalcitrance of biomass during conversion to biofuel. Owing to this fact, xylan biosynthesis pathway has been receiving increased attention (York and O'Neill 2008). Several CesA-like genes falling within the CAZy family GT2 glycansynthases have been implicated in biosynthesis of mannan, glucomannan, galactomannan, and xyloglucan backbone (Lerouxel et al. 2006; Suzuki et al. 2006; Liepman et al. 2007). IRX9 and IRX14 have been shown to play a role in xylan backbone synthesis (Pena et al. 2007; Brown et al. 2007; Lee et al. 2007). Recently, it was shown that PoGT4C, glycosyltransferase belonging to family GT47, is important to glucuronoxylan biosynthesis in hybrid

poplar, downregulation of which resulted in increased wood digestibility (Lee et al. 2009). It remains to be determined whether biosynthesis of hemicelluloses requires coming together of protein complexes and whether they are pathway-specific transcriptional regulators. Loss-of-function and gain-of-function poplar mutants are currently being evaluated to determine the roles of strong candidate genes in lignin, cellulose, hemicellulose biosynthesis pathways identified under the umbrella of the US Department of Energy (US DOE)-sponsored BioEnergy Science Center (BESC) to advance our understanding of the genetic control of wood characteristics.

Conversion of wood biopolymers to biodiesel

Genetically engineered trees and processes like CBP can significantly enhance second-generation biofuel production as a replacement for petroleum-based gasoline. An equally important challenge is the need to develop large volume, nonfood-based biodiesel. Although algae-based biodiesel has received substantial attention to address this need (Vasudevan and Briggs 2008; Chisti 2008; Ratledge and Cohen 2008), researchers have begun to also examine biological pathways that utilize lignocellulosic resources (Li et al. 2008; Meng et al. 2009). For example, the metabolic pathway leading to fatty acids starts the same way as the one that leads to alcohol; sugars are converted to pyruvate. From here, an aerobic anabolic process takes place that requires nicotinamide adenine dinucleotide phosphate (NADPH) to build up fatty acid chains from acetyl- and malonyl-coenzyme A (CoA), products of pyruvate decarboxylase and acetyl-CoA carboxylase. This reducing equivalent (NADPH) has to come from the substrate; consequently, some of the feedstock turns into CO_2 to provide energy (Ratledge and Wynn 2002; Ratledge 2004). Some engineered microorganisms, e.g., *E. coli*, can overproduce fatty acids (Lu et al. 2008) and even biodiesel directly (Kalscheuer et al. 2006); usually, however, strains build up triacylglycerols (TAGs) or lipids (oils and fats) that need to be transesterified with short-chain alcohols like methanol to gain fatty acid methyl esters (FAMEs; Ratledge and Wynn 2002).

Microbes that are capable to build up over 20% of their weight in lipids are called oleaginous (Ratledge and Wynn 2002). Some fungal and bacterial species are capable of achieving this from biomass components as recently reviewed (Li et al. 2008; Meng et al. 2009), turning lignocellulosics into oils for biodiesel. Fungi, yeasts, and molds utilize special enzymes like acetyl-CoA lyase and malic enzyme to run an NADPH and fatty acid-producing "metabolon" system that is unique to these oleaginous species. This peculiar cooperation between mitochondrium, ER membrane, and fatty acid synthase in the cytoplasm produces lipids as energy reserves (Ratledge and Wynn 2002; Zhang and Ratledge 2008) under unbalanced growth conditions like nitrogen (Ratledge and Wynn 2002) or phosphate (Wu et al. 2010) starvation while carbon is still abundant. Bacteria do not have this system and use polyhydroxy alkanoates (PHA) as reserves; however, a small group of actinomycetes build up significant TAG reserves and are under intense research recently (Alvarez and Steinbüchel 2002). An enzyme called wax ester synthase/acyl-CoA:diacylglycerol acyltransferase has been

Table 2 Several efficient TAG (or lipid)-producing speciesH

Species	Lipid yield (g/g substrate)	Productivity (g/l h)	Major fatty acids in total lipid (%)	References
Rhodosporidium toruloides	0.23	0.54	Palmitic acid (20) Stearic acid (14.6) Oleic acid (46.9) Linoleic acid (13.1)	Li et al. 2007
Cryptococcus curvatus	0.32	0.42	Np	Papanikolaou and Aggelis 2002
Candida sp. 107	0.37	0.4	Np	Papanikolaou and Aggelis 2002
Rhodococcus opacus PD630	0.15	0.38	Palmitic acid (30.8) Palmitoleic acid (9.9) Heptadecenoic acid (12.3) Oleic acid (24.4)	Voss and Steinbüchel 2001
Mortierella ramanniana	0.13[a]	0.17	Np	Hiruta et al. 1997
Trichosporon cutaneum	np	0.14	Np	Moreton 1988
Trichosporon fermentans	np	0.1	Np	Zhu et al. 2008

[a] Calculated from results, assuming that all substrate was consumed

np Not published

shown to be responsible for TAG formation instead of membrane lipid. This enzyme has recently been identified in multiple species (Wältermann et al. 2007) and cloned heterologously (Kalscheuer et al. 2006; Lu et al. 2008), resulting in oleagenecity even in *S. cerevisiae* (Kalscheuer et al. 2004). Genetic and metabolic engineering enabled researchers to modify *E. coli* to produce FAMEs directly utilizing xylene as carbon source (Steen et al. 2010). *Rhodococcus opacus* DSM 1069 has been reported to utilize lignin monomers, such as coniferyl alcohol, as carbon source (Eggeling and Sahm 1980), and in another study, *Rhodococcus* species were shown to be oleaginous (Alvarez and Steinbüchel 2002). Connecting waste lignin streams from biofuel production to lipid production is of great interest and has placed *Rhodococci* into the focus of research. These recent promising advances confirm the viability of biodiesel production from biomass, and Table 2 shows some promising strains with their productivity when grown on glucose as an alternative to ethanol production.

Conclusion

In summary, wood-based biofuels continue to provide a viable substitute to traditional gasoline and diesel. Advances in tree genetics and biological processing promise to further simplify and improve these conversion technologies, providing viable, renewable routes to today and tomorrow's transportation fuel needs.

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