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# Poplar as a feedstock for biofuels: A review of compositional characteristics

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Abstract: The growing demand for transportation fuels, along with concerns about the harmful effects of greenhouse gas emissions from the burning of fossil fuels, has assured a viable future for the development of alternative fuels from renewable resources, such as lignocellulosic biomass. The efficient utilization of these biomass resources is critically dependant on the in-depth knowledge of their chemical constituents. This, together with the desired fuel properties, helps tailor the chemical and/or enzymatic processes involved in converting biomass to biofuels. Hybrid poplars are among the fastest growing temperate trees in the world and a very promising feedstock for biofuels and other value-added products. Sequencing of the poplar genome has paved the way for tailoring new cultivars and clones optimized for biofuels production. Our objective is to review published research on the composition of the key chemical constituents of hybrid poplar species used for biofuels. Biomass yields, elemental composition, carbohydrate and lignin content and composition are some of the characteristics reviewed, with emphasis on lignin structure. Genetic modifications used to alter lignin content and composition, with the aim of improving biofuels yields, are also examined. © 2010 Society of Chemical Industry and John Wiley & Sons, Ltd

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## Introduction

The growing global energy demand and concerns about the negative effects of growing greenhouse gas (GHG) emissions from fossil fuels call for alternative energy sources, which are low cost, renewable and non-polluting. [1–5] One such renewable resource for producing different forms of energy is biomass. It has been a major source of energy for mankind since ancient times and presently contributes around 10–14% of the world's energy supply.[6] Biomass can be converted to different types of energy sources including heat, electricity, and liquid transportation fuels. It can also be used as a feedstock for chemicals production. As outlined in the USDA-DOE *Billion Ton* report, the agriculture and forestry reserves in the United States have the potential to address about one-third of the country's petroleum

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demand.[7] With increasing government, academic, and industrial research efforts on the production of biofuels and biomaterials from lignocellulosic feedstock, a few species have emerged as front-runners in this field. In the United States, these include hybrid poplar, switchgrass, Miscanthus, southern pine, willow, and corn stover.

The genus *Populus* comprises 25 to 35 species of deciduous plants native to the Northern Hemisphere. Common names used for the different species include poplar, aspen, and cottonwood. There is considerable genetic diversity within this genus and hybrids are readily produced to yield desirable traits. Poplar breeding mainly focuses on three native species: *Populus deltoides* (eastern cottonwood), *Populus balsamifera* (balsam poplar) and *Populus trichocarpa* (western black cottonwood); and two non-native species: *P. maximowiczii* (Asian black poplar) and *P. nigra* (European black poplar).[8] Hybrid poplars are among the fastest-growing trees in North America and are well suited for a variety of applications such as biofuels production, pulp and paper and other biobased products, such as chemicals and adhesives. Sequencing of the poplar genome has paved the way for tailoring new clones optimized for biofuels production.[9] Some key energy-related characteristics of lignocellulosic feedstock include cellulose, lignin, and hemicellulose content, bark content, moisture content, heating value, ash content and composition (inorganic elements present) and amount of extractives.[10,11] Other characteristics that impart good value to a bioenergy crop are drought tolerance, resistance to pests and insects, and the ability to produce high biomass yields on many different types of land. These characteristics can also be enhanced by genetic modifications. Studies on clonal variation in these properties in hybrid poplar have reported variations influenced by several factors, including changes in the cambium during growth, related to genetic background, water availability, insect and bacterial attacks, gravitopic effects, and other environmental influences.[12]

All processes used for the conversion of biomass feedstocks are sensitive to feedstock composition and quality to various extents. The specific gravity of wood and its lignin and cellulose contents have been proposed as prime targets for genetic modification.[10] The specific gravity of wood usually positively correlates with its cellulose content.[10] Reduction in lignin content is of greatest value as it improves enzymatic hydrolysis, which along with pre-treatment, is the most expensive component in the production of cellulosic ethanol. It typically results in a proportional increase in the cellulose content per unit mass.[10] Production of hybrids with desired qualities can be accomplished either via classic breeding techniques linked with marker aided selection and/or by genetic transformations. While genetic transformation can save time by bypassing the reproductive cycle and sometimes long generation intervals, a combination of the two complementary techniques is considered ideal.[11] Details of these techniques applied toward genetic improvement of poplar feedstocks can be found in articles by Dinus.[10,11]

Various studies have reported the results of chemical pre-treatments on poplar hybrids.[13–18] While most provide some compositional information on the starting feedstock, a review of the detailed compositional characteristics of poplar from a biofuels perspective is much needed. Ash content and composition, heating value, elemental ratios and proportion of lignin, cellulose, and hemicellulose are some of the broad compositional characteristics used to screen biomass feedstocks for biofuels applications. Other, in-depth compositional information that can be very useful in selecting the best feedstock for a particular conversion pathway are cellulose structure and degree of polymerization, hemicellulose composition, and the chemical nature and structure of lignin. In this review, both the broad and detailed compositional characteristics have been compiled for hybrid poplar species used for biofuels production. Where available, these compositional traits have been compared to those from other biomass feedstocks. Special emphasis has been given to the lignin fraction as successful implementation of a ‘biorefinery’ concept for poplar greatly depends on the utilization of the lignin as a value-added coproduct.[2,3]

## Poplar yield and chemical composition

### Poplar productivity in North America

Hybrid poplars are commonly classified as short-rotation woody crops and can be grown on forest lands or on economically marginal crop lands. Clonally propagated trees are harvested with conventional forestry equipment and delivered to processing facilities in the form of chips. Distribution maps of the genus *Populus* and the species *P. deltoides* and

*P. tremuloides* presented in Figure 1 show a very wide spatial distribution of hybrid poplar in the USA and Canada. Given the widespread distribution of poplar in the USA, suitable species or hybrids can be chosen for cultivation close to processing facilities in any region. Yields of first-generation hybrid poplar planted on croplands in the Lake States of the USA have been estimated to be in the range of 7.9 to 11.8 dry Mg $ha^{-1}$ $year^{-1}$. The reported yield is slightly lower on corn lands in Minnesota, with values ranging from 7.7 to 9.9 dry Mg $ha^{-1}$ $year^{-1}$.[8] The nominal yield (including moisture content at harvest) of hybrid poplar species in North America is estimated to be 14 Mg $ha^{-1}$ $year^{-1}$.[19] This is comparable to that of switchgrass (14 Mg $ha^{-1}$ $year^{-1}$) and much higher than corn stover (8.4 Mg $ha^{-1}$ $year^{-1}$) and wheatstraw (6 Mg $ha^{-1}$ $year^{-1}$).[20] In Quebec, yields of 17.3 Mg $ha^{-1}$ $year^{-1}$ were obtained without fertilizers or irrigation.[21] Upon maturity, poplar species can grow up to approximately 26 m in height and 60 cm in diameter. Dimensions of mature trees of common poplar species in North America are listed in Table 1.

**Table 1. Mature tree dimensions of common poplar species.[70]**

| Poplar species | Mature tree height (m) | Mature tree diameter (cm) |
|---|---|---|
| *P. tremuloides* Michx. | 15–18 | 30–61 |
| *P. balsamifera* L. | 18–24 | 30–61 |
| *P. deltoides* | 23–26 | 61–91 |
| *P. trichocarpa* | 14–18 | 30–61 |

## Heating values and elemental (C, H, N, O) composition

### Heating values

Heating value is the net enthalpy released upon reacting a material with oxygen under isothermal conditions. If water vapor formed during the reaction condenses at the end of the process, the latent enthalpy of condensation contributes to what is termed the *higher heating value (HHV).*

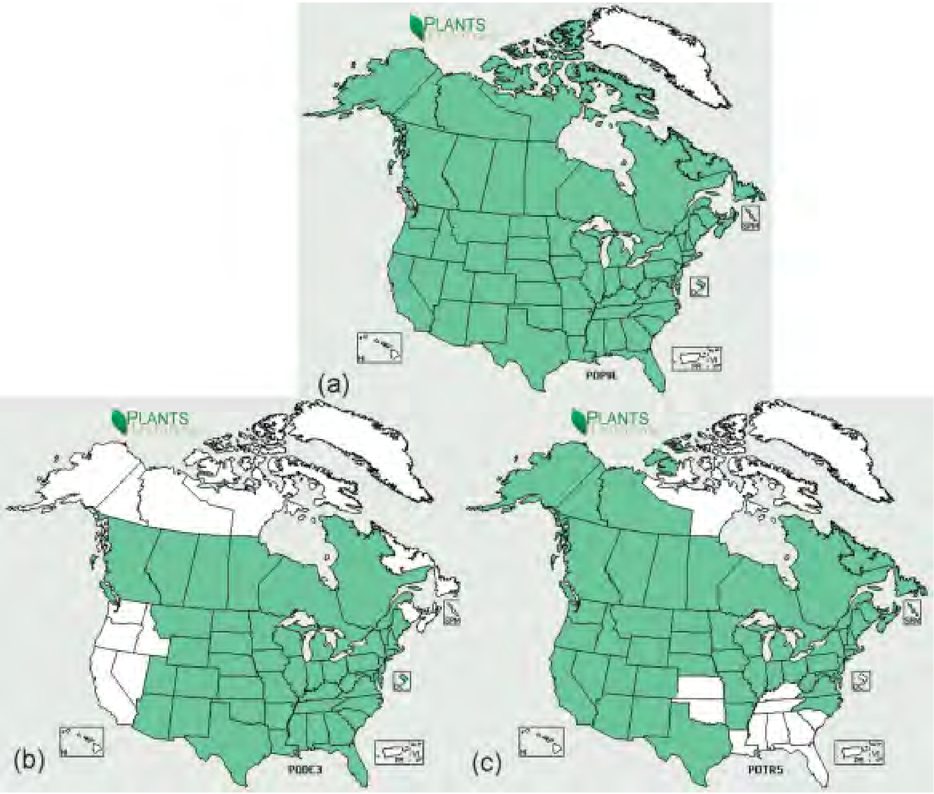


Figure 1. Distribution of poplar species in the USA and Canada.[22] (A) *Populus* (genus) (B) *P. deltoides*, (C) *P. tremuloides.*

**Table 2. Heating values (HHV) of hybrid poplar and other common biomass feedstocks.[20]**

| Biomass clone or species | Heating value (dry) (MJ/kg) |
|---|---|
| Hybrid poplar | 19.38 |
| *P. deltoides* | 16.26 |
| Black cottonwood | 15.00 |
| *P. tremuloides* | 13.50 |
| Ponderosa pine | 20.02 |
| Douglas-fir | 20.37 |
| Corn stover | 17.65 |
| Wheatstraw | 17.51 |
| Switchgrass | 18.64 |

These measurements are typically performed in a bomb calorimeter. Reported heating values (HHV) for hybrid poplar species are around 19 MJ/kg.[20,23] These values are comparable to other woody (e.g., oak, pine and Douglas-fir), herbaceous (switchgrass, Sudan grass) biomass feedstocks and agricultural residues (corn stover, wheatstraw, sugarcane bagasse; Table 2).

## Elemental composition

The major elemental composition of biomass on a gravimetric basis, which is commonly referred to as *ultimate analysis* is very important in performing mass balances on biomass conversion processes. These results can also be used to calculate empirical molecular formulae. Elemental compositions for hybrid poplar species compiled from the literature are given in Table 3. Data from a few other biomass feedstocks are also included for comparison. The sulfur content of poplar wood is low compared to wheatstraw and switchgrass (Table 3), which is advantageous in terms of strict environmental regulations limiting the sulfur content of transportation fuels. As expected, there is not much variation in the elemental composition of different poplar species.

## Ash content and inorganic element profiles

The inorganic elements present in biomass collective constitute its ash content and act as a waste stream during its conversion to biofuels and are the source of biochar and slagging during thermochemical conversion. Knowledge of the ash content and composition is essential regardless of the conversion pathway or end product. In addition, several studies have highlighted that soil productivity requirements may necessitate that this valuable inorganic resource be returned to the soils.[24,25] [20] Also, some inorganic elements, such as P, K, Ca, and Mg, act as macronutrients and a knowledge of their contents in the biomass can provide information on nutrient depletion of the soil, which can be used to maintain soil fertility in subsequent rotations.[26] A compilation of available data on ash content and selected inorganic element distributions of different hybrid poplar species and other softwood, hardwood and herbaceous biomass are given in Table 4. The data presented in Table 4 shows that while there is significant variation in ash content

**Table 3. Elemental (C, H, N, O, S) contents of poplar species.**

| Biomass clone or species | Ultimate analysis (% dry wt.) C | H | O | N | S |
|---|---|---|---|---|---|
| Hybrid poplar[20] | 48.45 | 5.85 | 43.69 | 0.47 | 0.01 |
| Poplar, DN 34[71] | 50.02 | 6.28 | 42.17 | 0.19 | 0.02 |
| Eastern cottonwood[72] | 50.29 | 6.45 | – | – | – |
| Hybrid poplar[73] | 50.20 | 6.06 | 40.40 | 0.60 | 0.02 |
| Hybrid poplar DN 34[23] | 51.73 | 4.47 | 35.11 | 0.24 | 0.03 |
| *P. deltoides*, Stoneville 66[23] | 49.65 | 5.85 | 41.88 | 0.08 | 0.05 |
| Corn stover[20] | 43.65 | 5.56 | 43.31 | 0.61 | 0.01 |
| Switchgrass[20] | 47.75 | 5.75 | 42.37 | 0.74 | 0.08 |
| Wheatstraw[20] | 43.20 | 5.00 | 39.40 | 0.61 | 0.11 |
| Ponderosa pine[20] | 49.25 | 5.99 | 44.36 | 0.06 | 0.03 |

Table 4. Ash content and inorganic elements (% dry weight) of poplar species and other biomass.

| | Ash | Inorganic elements (% dry wt.) | | | | |
|---|---|---|---|---|---|---|
| Biomass clone or species | (% dry wt.) | P | K | Na | Ca | Mg |
| Hybrid poplar[20] | 1.43 | | | | | |
| Caro[12] | 1.80 | 0.06 | 0.21 | 0.01 | 0.56 | 0.04 |
| DN 34[12] | 2.07 | 0.08 | 0.24 | 0.01 | 0.56 | 0.05 |
| DN 5[12] | 1.78 | 0.06 | 0.22 | 0.01 | 0.52 | 0.04 |
| DN 70[12] | 1.51 | 0.04 | 0.20 | 0.01 | 0.39 | 0.04 |
| DN 74[12] | 2.13 | 0.06 | 0.23 | 0.02 | 0.57 | 0.05 |
| NM 5[12] | 1.90 | 0.06 | 0.20 | 0.01 | 0.57 | 0.03 |
| NM 6[12] | 1.93 | 0.06 | 0.18 | 0.01 | 0.55 | 0.03 |
| CAFI high-lignin poplar[74] | 1.1 | | | | | |
| CAFI low-lignin poplar[74] | 0.8 | | | | | |
| NE 49[26] | 0.64 | 0.07 | 0.37 | | 0.46 | 0.06 |
| NE 245[26] | 0.80 | 0.08 | 0.31 | | 0.57 | 0.06 |
| NE 252[26] | 0.77 | 0.07 | 0.30 | | 0.64 | 0.05 |
| NE 279[26] | 0.60 | 0.09 | 0.37 | | 0.71 | 0.05 |
| NE 302[26] | 0.81 | 0.09 | 0.37 | | 0.55 | 0.06 |
| NE 350[26] | 1.20 | 0.08 | 0.37 | | 0.57 | 0.06 |
| NE 388[26] | 0.82 | 0.09 | 0.35 | | 0.63 | 0.05 |
| Willow (*Salix alba*)[12] | 2.29 | 0.49 | 1.83 | 0.15 | 6.76 | 0.48 |
| Oak[75] | | | 0.09 | 0.01 | 0.08 | 0.02 |
| Switchgrass[76] | 4.30 | 0.05 | 0.07 | 0.02 | 0.62 | 0.05 |

ranging from 0.6 to 2.7%, the distribution of inorganic elements shows very little variation among the different species. In general, the ash content of hybrid poplar clones is slightly higher than softwood biomass, but substantially (2× to 4×) lower than other biofuels feedstocks such as switchgrass, corn stover and wheatstraw.[20]

### Extractives content

Non-structural material is often removed from biomass prior to chemical analysis due to its potential interference with analytical techniques. This includes solvent-soluble, non-volatile compounds such fatty acids, resins, chlorophyll, waxes, etc., and usually comprises a minor proportion of biomass. For large-scale lignocellulosic biorefinery operations, however, extractives can be a potential source of value-added coproducts. The compounds present in the extractives fraction are a function of the solvent, which is usually ethanol, acetone, dichloromethane, or a mixture of ethanol/benzene. The ethanol and ethanol/benzene extractives content of some poplar species are presented in Table 5. Ethanol extractives include waxes and chlorophyll, whereas ethanol/benzene extractives also include low-molecular weight carbohydrates. The ethanol extractives content of poplar species is similar to corn stover and pine, but is much lower compared to that of switchgrass (Table 5). To avoid the use of large amounts of organic solvents on an industrial scale, the extractives fraction can be effectively isolated by using supercritical $CO_2$ or steam as the solvent.

## Cellulose, hemicellulose, and lignin in poplar species

### Relative proportions of cellulose, hemicellulose, and lignin

Cellulose, hemicellulose and lignin are the major biochemical components of lignocellulosic biomass.[1,2,3,5] Of the three, cellulose is most amenable to the production of ethanol and other higher molecular weight alcohols by its enzymatic hydrolysis to glucose followed by fermentation to ethanol

**Table 5. Extractives contents (% dry weight) of poplar species and other biomass feedstock.**

| Biomass species | Extractives content (% dry weight) |
|---|---|
| *P. tremuloides*[70] | 2.4[a] |
| *P. deltoides*[70] | 1.4[a] |
| *P. trichocarpa*[70] | 2.7[a] |
| CAFI high-lignin poplar[74] | 3.6[b] |
| CAFI low-lignin poplar[74] | 3.4[b] |
| Switchgrass[23] | 15.5[b] |
| Corn stover[23] | 3.9[b] |
| Monterey pine[23] | 2.7[b] |

[a] alcohol-benzene

[b] ethanol

by yeast or bacteria. Hemicellulose can also be converted to ethanol using a process similar to that used for cellulose, but modified to include hemicellulose-degrading enzymes such as xylanase and micro-organisms capable of fermenting pentose, as well as hexose sugars. Hardwood species and herbaceous plants usually have higher hemicellulose contents than softwoods.[1,2,3] Hemicellulose can also be utilized in the production of coproducts, such as furfural and acetic acid. The lignin fraction of biomass is also of interest in biofuels production as it is closely associated with cellulose and hemicellulose and it is also a useful biomaterial in its own right. The effective use of plants as a bioenergy feedstock is somewhat dependant on the extent of lignification of the cell wall. In the production of biofuels via a biological route, lignin is mostly utilized as an energy source for the pre-treatment stage and distillation of ethanol. Lignin can be used in a variety of industrial applications, however, and can also be converted to biodiesel or other liquid fuels.

The proportion of cellulose, hemicellulose, and lignin in a biomass feedstock is a very important criterion in determining its suitability as an economically viable feedstock and also in deciding on the optimum pathway for its conversion. Table 6 lists data from the literature on the proportion of cellulose, hemicellulose, and lignin in various poplar species and hybrids. Data from other commonly used biomass resources are also presented for comparison. Poplar species and hybrids have cellulose contents ranging from ~42 to 49%, hemicellulose from 16 to 23%, and total lignin contents from 21 to 29% (Table 6). The cellulose content of poplar is higher than that of switchgrass and corn stover and comparable to other hardwood feedstock such as eucalyptus, making it a desirable feedstock for the production of ethanol. It has higher lignin content than switchgrass or corn stover, however, which should be considered while designing pre-treatments and conversion strategies for poplar. Poplar

**Table 6. Proportion of cellulose, hemicellulose and lignin (as% dry weight) of poplar and other biomass.**

| Biomass clone or species | Cellulose (% dry wt.) | Hemicellulose (% dry wt.) | Lignin (% dry wt.) | | |
|---|---|---|---|---|---|
| | | | Total | Acid soluble | Acid insoluble |
| *P. deltoides*, Stoneville 66[23] | 42.2 | 16.60 | 25.6 | | |
| NM 6[18] | 48.95 | 21.70 | 23.25 | 20.95 | 2.30 |
| CAFI high lignin[74] | 43.80 | 20.40 | 29.10 | | |
| CAFI low lignin[74] | 45.10 | 21.50 | 21.40 | | |
| Caudina DN 34[71] | 43.67 | 19.55 | 27.23 | | |
| DN 182[23] | 45.52 | 20.75 | 23.58 | | |
| DN 17[23] | 43.65 | 23.24 | 23.07 | | |
| NC 5260[23] | 45.08 | 20.31 | 21.54 | | |
| Switchgrass[23] | 33.75 | 27.04 | 16.80 | | |
| *Eucalyptus saligna*[23] | 48.07 | 12.69 | 26.91 | | |
| Monterey pine[23] | 41.70 | 20.50 | 25.90 | | |
| Corn stover[23] | 37.12 | 24.18 | 18.20 | | |

**Table 7. Content of monosaccharides and uronic acids in poplar biomass.**

| | % dry weight | | | | | |
|---|---|---|---|---|---|---|
| Biomass clone | Arabinan | Xylan | Mannan | Galactan | Glucan | Uronic acid |
| Hybrid poplar[1] | 0.89 | 13.07 | 1.81 | 0.88 | 39.23 | 4.31 |
| NM6[18] | | 17.85 | 3.88 | | 48.95 | |
| CAFI high lignin[74] | 0.60 | 14.90 | 3.90 | 1.00 | 43.80 | |
| CAFI low lignin[74] | 0.50 | 17.80 | 1.70 | 1.50 | 45.10 | |
| Caudina DN 34[23] | 0.75 | 13.37 | 2.02 | 0.84 | 41.05 | 1.36 |
| *P. deltoides* Stoneville 66[23] | 0.60 | 13.40 | 2.00 | 0.60 | 42.20 | |
| DN 182[23] | 0.41 | 16.97 | 2.71 | 0.66 | 45.52 | |
| NC 5260[23] | 0.66 | 15.98 | 3.03 | 0.65 | 45.08 | |
| DN 17[23] | 0.54 | 18.71 | 3.25 | 0.74 | 43.65 | |

breeding programs may benefit from the selection of species with relatively lower lignin content. In case of cellulose and hemicellulose in poplar species, the distribution of individual monosaccharides is presented in Table 7. For the lignin fraction, the contents of acid soluble and insoluble lignin are given separately where available.

### Cellulose structure

Cellulose is a linear polymer of β (1→4) glucopyranosyl (Figure 2). The cellulose chain has a strong tendency to form intra- and inter-molecular hydrogen bonds by the hydroxyl groups on these linear cellulose chains, which stiffens the chains and promotes aggregation into a crystalline structure.[2,27] The degree of polymerization (DP) and crystallinity of cellulose can be a limiting factor in its enzymatic conversion to glucose.[28] The average number of β (1→4) glucopyranosyl units in the cellulose polymer is referred to as its degree of polymerization. The degree of polymerization of cellulose in natural materials can range from ~10 000 in cotton fibers and bacterial cellulose to 250–500 in regenerated cellulose fibers.[27] The ultrastructure of native cellulose (cellulose I) has been shown to possess an additional complexity in the form of two crystal phases: $I_\alpha$ and $I_\beta$.[29] Electron diffraction and nuclear magnetic resonance (NMR) studies have shown that cellulose $I_\alpha$ is an allomorph with triclinic unit cells, whereas cellulose $I_\beta$ is an allomorph with two-chain monoclinic units.[30,31] The relative amounts of $I_\alpha$ and $I_\beta$ has been found to vary between samples from different origins, with bacterial cellulose being rich in cellulose $I_\alpha$ and cellulose from higher plants being rich in $I_\beta$.[32] Most native cellulose also has varying degrees of amorphous cellulose, which lacks long-range order and is more reactive to chemical and enzymatic attack.

There is very limited data in the literature on cellulose DP and structure for woody biomass. Kumar *et al.*[15] have measured cellulose crystallinity directly on poplar samples (without isolating the cellulose) using wide-angle X-ray diffraction. Untreated poplar showed a crystallinity index (CrI), which is a measure of the proportion of crystalline cellulose, of 49.9. When this poplar feedstock was subjected to a variety of standard thermochemical pre-treatments, the CrI decreased slightly to 47.9 for the ammonia fiber explosion (AFEX), but increased for all others with the greatest increase seen for the flow-through acid treatment (CrI = 60.1). These authors also estimated the cellulose DP from viscosity measurements and obtained a DPv value of

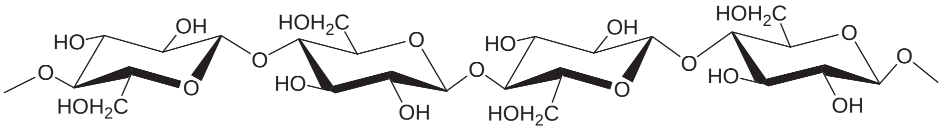


Figure 2. The structure of cellulose.

3500 for untreated poplar. Low pH pre-treatments of the poplar resulted in 65 to 70% reduction in DPv. Thus the enhanced cellulase hydrolyzability of pre-treated biomass may be attributed to the decreased cellulose chain lengths and greater availability of sugar reducing ends. Solid-state $^{13}$C CP/MAS NMR spectroscopy has also been used to determine the structure and crystallinity of cellulose isolated from lignocellulosic biomass.[32–35] Results from cellulose crystallinity and structure from CP/MAS NMR of cellulose from hybrid poplar are given in Table 8. Cellulose from poplar is 63% crystalline with cellulose $I_\beta$ as the predominant crystalline form. The less ordered amorphous region of poplar cellulose comprises 18% solvent inaccessible fibril surfaces. The intermediate *para*-crystalline form of cellulose also accounts for a significant proportion of poplar cellulose structure (Table 8). Crystallinity of poplar cellulose is comparable to that from Loblolly pine, but about 20% higher than switchgrass (Table 8). The amorphous region of switchgrass cellulose is mostly in the form of inaccessible fibril surfaces, however, which may hinder enzymatic hydrolysis.

The differences in poplar cellulose crystallinity between X-ray diffraction and NMR are due to the fact that different techniques are utilizing different principles to evaluate crystallinity.[36] Significant natural variation in cellulose crystallinity and ultrastructure (as determined by NMR) was observed in 18 poplar samples collected from trees of diverse ages/establishment cohorts over a relatively small (265 km) geographical range.[35] Cellulose crystallinity in these samples ranged from 54 to 68% and did not exhibit any discernible relationship to the diameter at breast height (DBH) of the tree, which is used as an indicator of tree age and maturity. These results suggest that the effects of biological considerations versus environmental growth factors on the resulting cellulosic ultrastructure of poplar cannot be readily delineated at this time.

## Hemicellulose composition

Hemicelluloses are branched polymers of low molecular weight and are composed of a relatively small number of sugar residues. The main hemicellulose of hardwoods, including poplar species, is *O*-acetylated 4-*O*-methyl-glucuronic acid xylan or glucuronoxylan (Figure 3).[2,37,38]

Hemicelluloses are typically extracted from biomass using a series of alkaline extractions that hydrolyze the ester linkage and remove them from the lignocellulosic matrix.[37,39] In a paper mill, hemicelluloses can be pre-extracted from wood with hot water or steam prior to kraft pulping and in the future may be utilized as adhesives, thickeners, stabilizers, and emulsifiers. The hemicellulose fractions of hybrid poplar species are mainly composed of glucuronoxylan, which can be readily modified to produce xylitol, a sugar substitute.[39] On average, poplar woodchips contain ~20% hemicellulose (Table 6). A comprehensive source of information on the hemicellulose in hardwoods is Willför *et al.*[38] who analyzed the polysaccharides present in the sapwood and heartwood of 11 hardwood species. Data from four poplar species – *P. deltoides x nigra*, *P. grandidendtata*, *P. tremula* and *P. tremuloides* – were included in their survey. Xylans (15.9 to 22.4%) are the major hemicellulose in all the poplar species in their study, followed by mannans (0.9 to 3.4%). 4-*O*-methyl-glucuronic acid (4-*O*-MeGlcA; 2.2 to 2.8%), galacturonic acid (2.3 to 2.8%) and minor amounts of glucuronic acid (0.1 to 0.3%) have been identified as the uronic acids present in poplar.[38] Gabrielii *et al.*[37] characterized hemicellulose isolated from *P. tremula* using alkali extraction combined with ultrafiltration. On the basis of two-dimensional COSY and HMQC NMR results, its structure was identified as a linear (1→4)-β-linked D-xylose main chain with a 4-*O*-methyl-α-D-glucuronic acid substituting the 2-position of approximately every eighth xylose unit. The weight average and number average molecular weights

**Table 8. Cellulose crystallinity and structure determined from CP/MAS $^{13}$C NMR.**

| (%) | Crystallinity | $I_\alpha$ | $I_{\alpha+\beta}$ | para - crystalline | $I_\beta$ | Accessible fibril surfaces | Inaccessible fibril surface |
|---|---|---|---|---|---|---|---|
| *P. trichocarpa x deltoides*[a,35] | 63 | 5.0 | 14.2 | 31.1 | 19.8 | 10.2 | 18.3 |
| Loblolly pine[34] | 63 | 0.1 | 30.7 | 24.8 | 6.9 | 33.1 | 15.6 |
| Switchgrass Alamo[77] | 44 | 2.3 | 8.8 | 27.3 | 4.5 | 5.7 | 51.3 |

[a] values in the table represent the average of 5 samples.

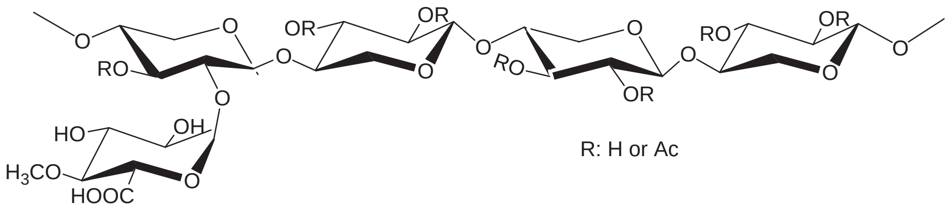


Figure 3. Structure of glucuronoxylan, the predominant hemicellulose in hardwoods.

of this hemicellulose sample after conversion to acetoxypropyl xylan were 73 100 g/mol and 48 000 g/mol. This results in a polydispersity index of 1.5. However, poplar hemicellulose fractions extracted using sequential extractions with increasing concentrations of NaOH (1.5 to 8.5%), had values of polydispersity index ranging from 5.1 to 8.0.[39] In these samples, the number average molecular weight ranged from 4910 to 7580 g/mol, while the weight average molecular weight varied between 38 360 to 42 230 g/mol. Thus, the molecular weight results for hemicelluloses seem to depend, in part, on the isolation procedure employed. The degree of polymerization of hemicelluloses is usually lower than cellulose and ranges from 50 to 300.

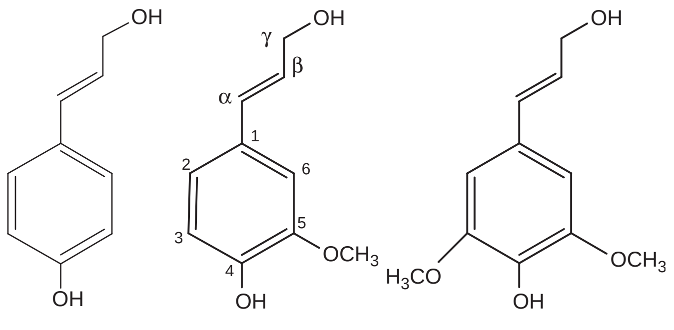
Lee *et al.*[40] studied the effects of down-regulating the expression of the poplar glycosyltransferase (*PoGT47C*) gene, which is known to play a role in the biosynthesis of glucuronoxylan, in hybrid poplar (*P. alba x tremula*). This resulted in a drastic reduction in secondary cell wall thickness, deformation of vessels, and reduced amount of glucuronoxylan in the wood. The transgenic wood was also found to yield more glucose by cellulase enzymatic hydrolysis than the wild-type wood. Cellulose and glucuronoxylan form a network in secondary cell walls, however, and a severe reduction in the hemicellulose content can lead to a drastic alteration in cellulose deposition and change the organization of secondary cell walls.[40] Thus, a controlled reduction in glucuronoxylan content of poplar or other hardwoods by genetic modification could provide a pathway for reducing biomass recalcitrance to enzymatic saccharification.

## Chemical nature and structure of lignin

### Overview of lignin structure and analytical techniques

Lignin is an amorphous, cross-linked, and three-dimensional phenolic biopolymer. Lignins comprise the second most abundant class of polymers in the biosphere after cellulose.[41] Their biosynthesis arises from the polymerization of three types of phenylpropane units as monolignols: coniferyl, sinapyl, and *p*-coumaryl alcohol (Figure 4). The relative abundance of these units depends on the contribution of a particular monomer to the polymerization process. The exact mechanism of lignin polymerization and biosynthesis has been hotly debated in the literature and details of this complex pathway can be found in review articles by Lewis,[42] Boerjan *et al.*[43] and Ralph *et al.*[41] Hardwood lignin is composed mainly of syringyl (S) and guaiacyl (G) units with minor amount of *p*-hydroxyphenyl (H), whereas softwood lignin is composed mainly of guaiacyl units and trace amounts of H.[2,3,41,42,43,49]

Figure 5 shows some common hardwood lignin inter-unit linkages. β-*O*-4 (β aryl ether) linkages are the most frequently occurring inter-unit linkage and are also the ones most easily cleaved by chemical processes such as pulping and biomass pre-treatments. The other linkages β-5, β-β, 5-5, 4-*O*-5 and β-1 are all more resistant to chemical degradation.[43] Hardwood lignins with a higher proportion of S units have fewer β-5, 5-5 and 4-*O*-5 linkages than softwood lignin with more G units.[41]



*p*-coumaryl alcohol (H) Coniferyl alcohol (G) Sinapyl alcohol (S)

Figure 4. Three building blocks of lignin.

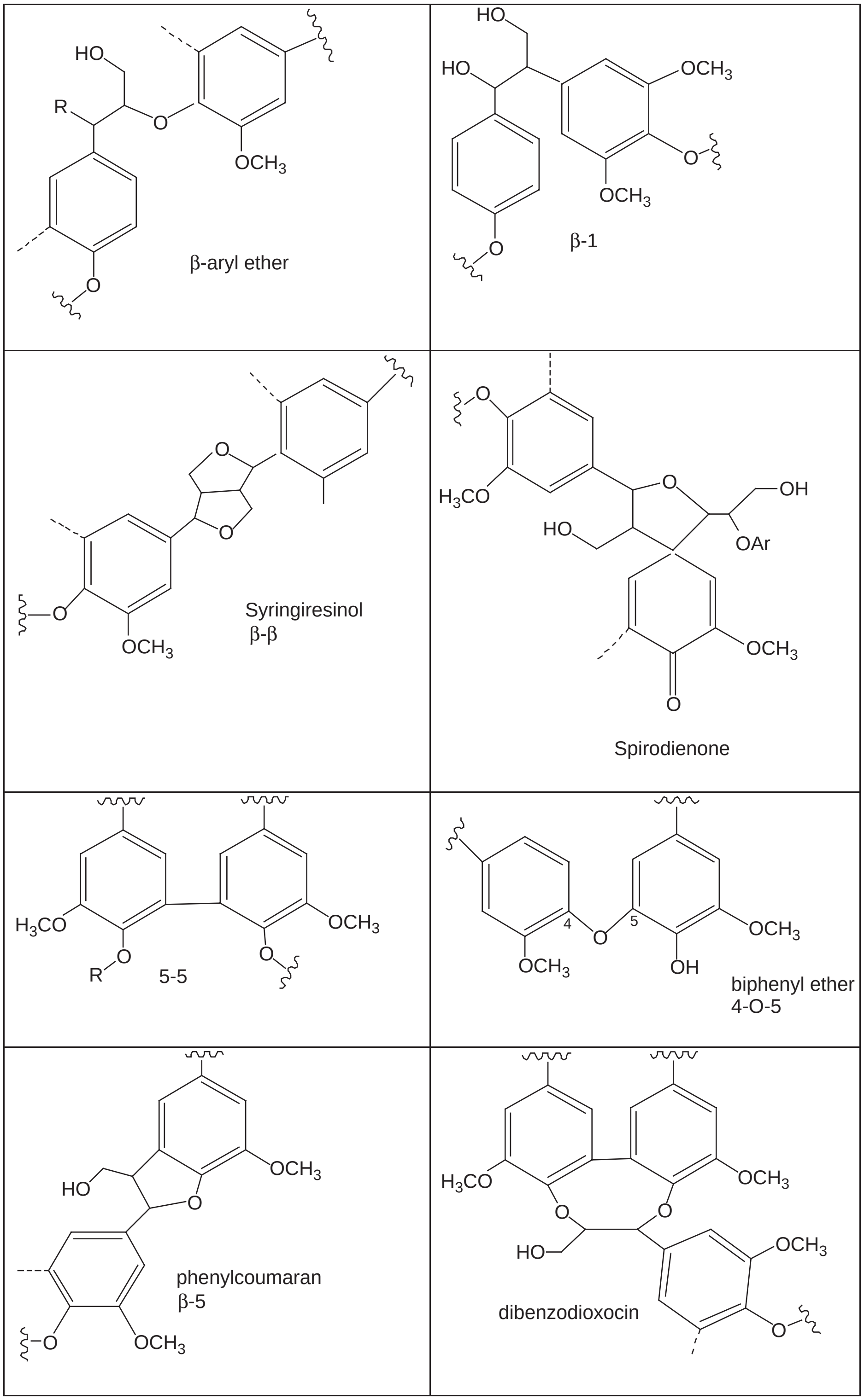


Figure 5. Hardwood lignin sub-unit structures.

Wet chemistry techniques, such as thioacidolysis and nitrobenzene oxidation, coupled with gas chromatography have been traditionally used to study lignin structure. While these methods can be very precise for specific functional groups and structural moieties, each technique can only provide limited information and does not give a general picture of the entire lignin structure. The thioacidolysis procedure cleaves β-*O*-4 linkages in lignin, giving rise to monomers and dimers which are then used to calculate the S and G content. Similar information can be obtained using nitrobenzene oxidation, but it can lead to overestimating S/G ratios.[44] In general, the S/G ratio of lignin is a good indicator of its overall composition and response to pulping and biomass pre-treatment. S/G ratios of lignin from different poplar species are summarized in Table 9. Differences can be seen based on the measurement technique as well as poplar species, but in general the S/G ratio ranges from 1.3 to 2.2. This is similar to that from the hardwood eucalyptus, but higher than herbaceous biomass switchgrass and Miscanthus. This is to be expected given the higher H contents in grass lignin. Recently, Bose *et al.*[45] used an optimized nitrobenzene oxidation method to determine the S/G ratios of 13 poplar samples from two different sites and obtained values ranging from 1.01 to 1.68. Further, these samples showed a linear correlation ($R^2 = 0.85$) between decreasing lignin content and increasing S/G ratios. The correlation was stronger ($R^2 = 0.93$) in samples from a single site suggesting a dependency on geographic location.

The advantage of spectroscopic methods over degradation techniques is their ability to analyze whole lignin structure and directly detect lignin moieties. The development of quantitative $^{13}C$ NMR for lignin analysis[46] was an important advance in lignin chemistry. Multidimensional NMR spectroscopy has also been successfully utilized to elucidate details of lignin structure.[47] While two-dimensional (2D) NMR can help provide unambiguous structural assignments, performing quantitative 2D NMR experiments requires special precautions and is typically only semi-quantitative at best.[48] A combination of quantitative $^{13}C$ and 2D HMQC NMR has been shown to provide comprehensive structural information on lignin from a variety of sources.[48,49] For example, 80% of the side chains of eucalyptus lignin were estimated at the structural level using these methods. Other NMR techniques, such as phosphitylation of lignin hydroxyl groups, followed by $^{31}P$ NMR, $^{1}H$ and HSQC NMR can also yield valuable structural information. The most frequently used procedure to isolate lignin for NMR analysis is ball milling wood to a fine meal, followed by lignin extraction with aqueous dioxane. This milled wood lignin (MWL) is regarded as being fairly similar to native lignin in wood.

With the advent of transgenic plants, higher throughput methods are being developed for rapid screening of bioenergy feedstocks. These include near-infrared (NIR) reflectance spectroscopy,[50] pyrolysis molecular beam mass spectrometry (pyMBMS),[51] Fourier transform infrared spectroscopy,[52] a modified thioacidolysis technique,[53] and whole cell NMR after dissolution in ionic liquids.[54] Information on some structural characteristics of lignin, such as S/G ratios, can be rapidly obtained using these methods. The average S:G:H ratio of 104 poplar lignin samples, determined using the modified thioacidolysis technique, was 68:32:0.02.[53] Recently, Hedenstrom *et al.*[55] applied multivariate chemometric analysis on 2D HSQC NMR data from poplar whole cell samples. Results from this technique readily confirmed known structural differences between poplar tension wood and normal wood, such as higher cellulose and lower lignin, xylan, and mannan content in tension wood. Within the lignin fraction, tension wood samples showed higher relative amounts of S and H and lower G. When this analysis was applied to wild-type poplar versus a transgenic line down-regulated in pectin methylesterase activity, the subtle differences in cell wall composition were seen in results from chemometric analysis of the NMR spectra. The authors concluded, however, that interpretation of this statistical information requires further investigation.[55]

## Structure of poplar lignin from NMR spectroscopy

Structural information obtained from NMR spectroscopy of lignin isolated from hybrid poplar species is summarized in this section. Results from quantitative $^{13}C$ NMR of hardwood lignin are integrated and the area of the aromatic region from 162.5 to 102 ppm is set equal to 6.0 (representing the six carbons in each aromatic ring and assuming negligible contributions from vinylic C).[49] All other peak areas are reported based on this normalization, in terms

**Table 9. S:G ratios of lignin from poplar and other biomass.**

| Biomass clone or species | Lignin S:G | Measurement technique |
|---|---|---|
| *P. tremuloides* | 0.65 | $^{31}$P NMR[60] |
| Hybrid poplar | 1.51 | Thioacidolysis[59] |
| Balsam poplar | 1.28 | Thioacidolysis[78] |
| Poplar | 1.74 | $^{13}$C NMR[79] |
| *P. tremula* x *alba* | 1.51 | Thioacidolysis[56] |
| Aspen | 1.40 | Thioacidolysis[80] |
| *P. tremuloides* | 1.86 | Thioacidolysis[44] |
| *P. alba* x *tremula* | 2.15 | Thioacidolysis[53] |
| *P. alba* x *tremula* | 2.19 | Modified thioacidolysis[53] |
| Switchgrass | 0.80 | $^{13}$C NMR[81] |
| *Eucalyptus grandis* | 1.72 | $^{13}$C NMR[49] |
| *Miscanthus* x *giganteus* | 0.84 | $^{13}$C NMR[82] |

of the number of a particular moiety per aromatic ring or per 100 aromatic rings. $^{13}$C NMR results from lignin isolated from two clonal aspen (*P. tremuloides*) samples from Stewart *et al.*[44] are summarized in Table 10. Some of the side chains quantified in this poplar lignin include, β-*O*-4, β-1, pinoresinol/syringoresinol and minor amounts of dibenzodioxocin and their amounts are given in Table 10. The $^{13}$C spectra from these poplar lignin samples do not show carbonyl peaks above 170 ppm implying negligible amounts of non-conjugated CO, α-CO, and vanillin moieties. Based on the lack of carbonyl signals, the oxygenated carbon functionalities (90 to 58 ppm) were inferred to be solely β-*O*-4 linkages. Also, the lack of signal at 87 ppm in the acetylated lignin spectra, indicates the absence of phenylcoumaran structures. Thus the integral in the region from 50 to 48 ppm, which is usually ascribed to phenylcoumaran and β-1structures, in this case represents only β-1 structures. For these poplar clones, the frequency of β-1 structures was ~10/100 Ar. In the aromatic region, methine (C-H) carbons in the 125–103 ppm range represent ring atoms substituted with carbon and oxygen. For these poplar clones, 206 C-H/100 Ar and 202 C-H/100 Ar were calculated from NMR spectra. Signals due to oxygenated aromatic carbons appear between 160 to 141 ppm and are used to estimate the syringyl content, which in this case is ~200/100 Ar. Some structures such as dibenzodioxocin, which are minor components of hardwood lignin, may be difficult to identify or be overestimated due to overlap with other peaks, in quantitative $^{13}$C NMR spectra. The degree of condensation of the two poplar lignin samples are calculated from the NMR data to be 7/100 Ar and 2/100 Ar.[44] The poplar clone with a lower degree of condensation and higher β-*O*-4 content (which also implies a higher syringyl content) in its lignin, showed higher pulp yields.[44] A similar correlation should hold good for lignin removal during thermochemical pre-treatments used in the process of converting biomass to ethanol.

**Table 10. Lignin structural information calculated from quantitative $^{13}$C NMR data.[44]**

| Structure | Number per 100 Ar<br>*P. tremuloides* Michx. 10-1 | <br>*P. tremuloides* Michx. 16-2 |
|---|---|---|
| β-1 | 10 | 9 |
| Pinoresinol | 8 | 9 |
| Dibenzodioxocin | 0 | 1 |
| β-*O*-4 | 56 | 68 |
| Methoxyl groups | 133 | 142 |
| Side chains | 306 | 304 |
| *p*-hydroxyphenyl | 11 | 10 |
| Oxygenated aromatic | 201 | 200 |

Stewart *et al.*[56] studied lignin from hybrid poplar (*Populus tremula x alba*) using a combination of $^{13}$C-$^{1}$H HMBC NMR, which is particularly useful in detecting long-range correlations between C and H, and short-range $^{13}C$-$^{1}H$ HSQC NMR. Although these spectra were not quantitative, they provided many structural details including the presence of phenylcoumaran units derived from both sinapyl and coniferyl alcohol coupling reactions, resinol units derived from S and arylglycerol from S. Also present were beta aryl ether, phenylcoumaran, resinol, *p*-hydroxybenzoate, and spirodienone groups.[56] Ammalahti *et al.*[47] applied 3D HMQC-HOHAHA NMR to the structure elucidation of poplar lignin. This method can help in definitive assignment of NMR signals to lignin structural units; however it is not routinely used, as the lignin samples need to be $^{13}C$ enriched in order to perform these experiments within a reasonable time frame. Some specific side chains structures in poplar lignin which could be identified using this technique are non-cyclic α-aryl ethers and *cis*- and *trans*-dibenzodioxocin, whose presence in hardwood lignin has been debated. The $^{1}H/^{13}C$ signals and assignments of some other side-chain structures identified in poplar lignin by 3D NMR are: α, β, γ carbons of phenylcoumaran (δ 3.80/50.4); α carbon of resinol (δ 4.72/85.8); side-chain of *trans*-dibenzodioxocin (δ 4.14/82.5); and $H_\beta$ of α-carbonyl side chain (δ 5.4/82).[46]

Hydroxyl groups in lignin can be quantified with $^{31}P$ NMR spectroscopy following the phosphitylation of the sample with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) or 2-chloro-1,3,2-dioxaphospholane (Figure 6).[57,58] The results of this analysis are usually presented as mmol OH/g lignin or mmol OH/$C_9$.

One particular advantage of this method, especially for hardwood lignin, is the detection and quantification of *p*-hydroxyphenyl (H) units. Lignin from poplar has been analyzed using $^{31}P$ NMR spectroscopy.[57–59] Ball-milled enzyme extracted lignin from *P. deltoides* was found to have 0.20 moles/$C_9$ phenolic OH groups, 1.18 moles/$C_9$ aliphatic OH, and 0.02 moles/$C_9$ COOH groups.[57] $^{31}P$ NMR data from Wu and Argyropoulos[60] on *P. tremuloides* and Akim *et al.*[59] on control and transgenic poplar lignin are summarized in Table 11. CAD and COMT down-regulation resulted in lignin with a lower amount of aliphatic OH and a higher condensed phenolic OH content than the control poplar lignin (Table 11).[59]

## Genetic modification of lignin content and composition in poplars

Genetic modifications that result in a reduction in lignin content are among the most common techniques employed to improve poplar feedstock quality. Most research involves manipulation of enzymes catalyzing the synthesis and inter-conversion of lignin precursors. Early research in this field was conducted on phenylalanine ammonia lyase (PAL), the enzyme at the gateway for carbon entry to lignin biosynthesis.[11] Emphasis has been switched to enzymes functioning later in lignin biosynthesis due to concerns over adverse effects of modifying PAL activity on other pathways. The genes common to the latter path of the lignin synthesis pathway generally affect the distribution of H, G and S lignin units.[56] Davison *et al.*[61] studied the effects of varying the S/G ratio and lignin content of hybrid poplar on the xylose release during dilute acid hydrolysis. This study was performed on poplar clones with natural S/G variation (from 1.8 to 2.3) and differences in lignin content (from 22.7 to 25.8%). Results from statistical analysis showed that a small decrease in S/G ratio resulted in a statistical increase in xylose release after dilute sulfuric acid hydrolysis. While the effects of lignin content were not statistically significant, an increase in lignin content led to a decrease in the xylose yield. Overall, the sample with a combination of low S/G ratio (1.8) and low lignin content

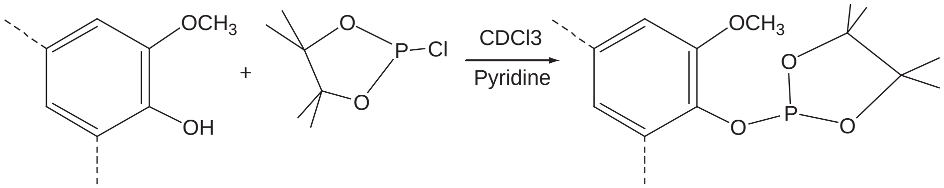


Figure 6. Derivatization of phenolic groups with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP).

**Table 11. Hydroxyl group contents of poplar lignin (mmol/g) calculated from quantitative $^{31}$P NMR.**

| Lignin source | Aliphatic OH | Phenolic OH | | | | Carboxylic acids |
|---|---|---|---|---|---|---|
| | | Condensed | Non-condensed | | | |
| | | | H | G | S | |
| Hybrid poplar[57] | 5.72 | 0.13 | 0.20 | 0.25 | 0.61 | 0.06 |
| 70% CAD down-regulated poplar[59] | 5.23 | 0.16 | 0.21 | 0.25 | 0.63 | 0.16 |
| 90% COMT down-regulated poplar[59] | 5.06 | 0.15 | 0.20 | 0.09 | 0.65 | 0.06 |
| *P. tremuloides*[60] | 4.53 | 0.22 | 0.17 | 0.37 | 0.24 | 0.14 |

(22.7%) showed the highest xylose release resulting from dilute acid hydrolysis.

Suppression of caffeate *O*-methyltransferases (COMT) which catalyses the ortho-methylation of 5-hydroxyferulate to sinapate, did not result in a reduction in lignin content but syringyl/guaiacyl ratios were reduced.[62] The enzyme cinnamyl alcohol dehydrogenase (CAD) catalyzes the last step in lignin precursor biosynthesis: the conversion of aldehydes to alcohols. Modification of CAD activity has been reported to have varying effects on poplar species. While some researchers observe 10–15% lower lignin contents and lower S/G ratios,[63] others report effects such as incorporation of aldehydes in lignin,[64] increased frequency of free phenolic groups, [65] and altered coloration of the xylem.[64,65] Another enzyme, whose activity has been successfully modified is ferrulate 5-hydroxylase (F5H), which is a key enzyme involved in synthesizing sinapyl alcohol and ultimately S lignin moieties. This enzyme affects the partitioning between the two major lignin precursors – coniferyl and sinapyl alcohols –and determines the S/G ratios of the resulting lignin. Overexpression of F5H enzyme resulted in transgenic poplar species with up to 95 mol% syringyl groups (as compared to 65 mol% in the control sample).[56] Further, while the total lignin content remained same, the transgenic poplar trees displayed higher levels of acid soluble lignin and lower Klason lignin content.

Genetic modifications to alter lignin content can also result in increased cellulose content. The 4-coumarate 3-hydroxylase (4CL) enzymes convert 4-coumarate, caffeate, ferulate, 5-hydroxyferulate and sinapate to their respective thioesters.[10] Two different 4CL genes, *Pt4CL* and *Pt4CL2* have been identified in *P. tremuloides*.[66] Of these two, *Pt4Cl* is mainly involved in lignin biosynthesis and genetic transformation with its antisense constructs yielded trees with 45% reduction in lignin content accompanied by a 15% increase in cellulose contents.[66] There were no changes in lignin structure and composition, or xylem coloration as seen in some other genetic modifications. Genetic transformation of poplar to increase cellulose content, although not as widespread as decreasing lignin content, has also been the focus of research efforts. Loopstra *et al.*[67] isolated and sequenced a family of cellulose synthase (celA) genes from poplar. Transgenic *P. tremuloides* with a celA sense construct has been found to have higher growth rates.[10] Park *et al.*[68] overexpressed the xyloglucanase enzyme in *Populus alba*, which led to increases in cellulose content, stem growth, and specific gravity.

## Single tree variability in lignin content and composition

Obtaining a representative sample is vital to all biomass compositional analysis. In case of woody biomass, it is important to consider compositional variability within the tree. The position at which a tree is sampled could have a great influence on biomass compositional characterization, especially when using rapid characterization techniques like NIR spectroscopy. Typically, for fully grown trees, wood cores are taken at DBH or approximately 1.3 m. Sykes *et al.*[69] studied the variability in lignin content and S/G ratios in poplar clones from samples taken from different heights and from different growth rings at a particular height. Results from pyMBMS were used to determine wood properties and principal component analysis of the data indicated minimal correlation of lignin content with sampling height. S/G ratios increased slightly (from 1.6 to 2.0) from the pith outwards as the number of rings increased. These results show that when estimating poplar lignin properties, the ring or rings chosen will have a larger effect than the sampling height.[69]

## Summary and conclusions

Hybrid poplar species are a promising feedstock for the production of bioethanol, thermal energy, and pulp. The elucidation of the poplar genomic sequence and the relative ease of its genetic manipulation have led to the development of transgenic poplar clones with enhanced chemical properties for their efficient conversion to biofuels. In North America, hybrid poplar species have high productivity, even in marginal lands, and their widespread growth area makes them suitable for cultivation in almost all regions of the USA. This provides flexibility in locating crop stands close to future biorefineries. In general, poplar has high cellulose content, low amounts of ash and extractives, and moderate lignin and hemicellulose contents. These properties make it a desirable feedstock for the production of ethanol and other biobased material. The lignin in poplar mostly comprises syringyl units, rendering it more labile to the chemical pretreatments used for biomass to biofuels conversion. With the proper choice of pre-treatments, which result in less degradation, the lignin in poplar can also be a valuable product, rather than just an energy source. For example, the lignin recovered during the ethanol organosolv pre-treatment can act as an antioxidant or as a precursor for the production of chemicals, such as vanilla, phenol or ethylene. The hemicelluloses can either be produced as a coproduct (for the production of adhesives, thickeners, emulsifiers, and sugar substitutes) or fermented to ethanol. With the advent of genetically modified micro-organisms capable of fermenting pentose as well as hexose sugars, the utilization of the hemicellulose fractions of poplars has become more efficient, thus making the process of their conversion to bioethanol even more economically attractive.

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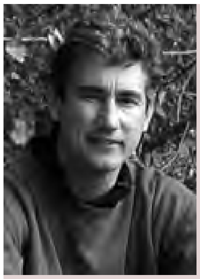

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