BIOENERGY PROPERTIES OF JUVENILE HYBRID POPLARS AND THEIR PARENT SPECIES

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Abstract. Bioenergy properties of poplar species *Populus trichocarpa* (PT), *Populus deltoides* (PD), and their hybrid were evaluated. Hybrid poplar trees from the cross between PT and PD presented different anatomic, physical, chemical, and thermal properties from their parent species. Anatomic results tended to suggest that hybrid poplar, with fewer vessels per unit area, had more resemblance to PT. Extractive content ranged from 10.64-11% for PD, PT, and first-generation hybrid poplar, whereas it varied from 8.8-9.5% for backcross offspring (BC2-BC5). PD had the greatest average lignin content of 25.6% followed by first-generation offspring and backcross offspring with lignin content of approximately 25%. Holocellulose content of hybrid poplar species was higher than that of their parent species. Observed stem/stump proximate results ranged from 72-74.7%, 25-28%, and 0.80-1.7% for volatile matter, fixed carbon, and ash content, respectively. Heating values observed along the stem were slightly higher than at the stump, ranging from 7498-8356 kJ. TGA-FTIR analysis indicated that H_2, CO_2, CH_4, and CO were the dominant gaseous components from wood pyrolysis.

Keywords: Anatomy, bioenergy, hybrid poplar, TGA-FTIR, thermal and chemical properties.

INTRODUCTION

Demand for bioenergy in the US is growing because of a concerted effort to decrease the nation's carbon footprint and dependency on fossil fuels. Currently, woody biomass, the most abundant naturally occurring resource, is a potentially important feedstock for production of various forms of bioenergy. Benefits of using biomass as feedstock for bioenergy include decrease in use of nonrenewable fuels, less dependency on foreign fuels, stabilization of income in rural areas, and decreased carbon emissions (Office of Technology Assessment 1993). Biomass has been targeted to replace 30% of fossil fuel used for transportation in the US by 2030 (DOE 2010).

The challenge with using biomass for transportation fuel production is caused by the recalcitrant nature of lignin. Cellulase enzymes produced from a host of microorganisms can be used to hydrolyze wood into fermentable sugars provided an appropriate pretreatment is used.

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However, enzyme productivity is limited by lignin content in pretreated wood, and thus there is a need to genetically engineer biofuel feedstock with naturally low lignin content. Poplar trees (native cottonwood, aspen species, and its hybrids) are suitable candidates for genetic improvement for bioenergy feedstock production because of their modest genome size (Perlack et al 2005). Hybrid poplar has a fast growth rate and high biomass yield of about 18,144 kg per 0.01 km^2 per year (Stanton et al 2002). Hybrid poplar also thrives on a wide range of conditions, can be harvested throughout the year with minimum investment in storage facilities (Wright 1994), and has comparable greenhouse gas emissions to other cellulosic crops because of less tillage and cutting (Adler et al 2007).

With the goal of examining the bioenergy potential of hybrid poplars and their parents, the objectives of this study were to 1) assess the anatomical, physical, and thermochemical-related properties of *Populus trichocarpa* (PT), *Populus deltoids* (PD), and resulting hybrids; and 2) compare bioenergy potentials among these poplar species in the central Appalachian region.

MATERIALS AND METHODS

Materials

Poplar trees used in this study (PT and PD) were established from vegetative cuttings at the West Virginia University Agronomy Farm. The plant material consisted of a single interspecific family produced from a pseudobackcross crossing design (Fig 1). Flowering branches were collected from female PT tree clone 93-968 from the State of Washington. Pollen was collected from a male PD clone, number ILL-101, and used for crosses in a greenhouse setting (Stanton and Villar 1996). This produced a first-generation (F1) hybrid, clone 52-225 (clone F1), which has been used extensively in commercial plantations (Van Oosten 2000). To dissect factors contributing to hybrid vigor in this commercial clone and genes that control lignin composition and its recalcitrance, F1 hybrid was backcrossed to a different PD tree, clone D124, from Minnesota (Riemenschneider et al 2001). This produced the pseudobackcross family 52124 (collectively referred to as clone BC1 in this article), which was used for establishing the plantation and stool bed at West Virginia University.

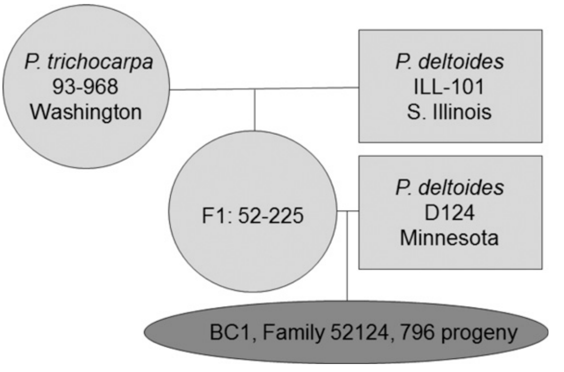

Figure 1. Crossing design for production of quantitative trait locus (QTL) mapping pedigree. Female clone 93-968 was crossed to *P. deltoides* male clone ILL-101 to produce F1 tree clone 52-225, a female tree. This tree was then crossed to another *P. deltoides* male clone, D124, to produce 796 progeny comprising family 52124.

A randomized complete block design with four replicates was used in this study. Two main factors considered were genotype and wood sample location. There were five genotypes, all of which were part of a two-generation pedigree: PD1, PD2, PT1, F1, and BC (BC1-5). The second source of variation (block) was location sample was taken from in the tree (ie stem or stump). Five backcross hybrid poplars (five different trees, named BC1, BC2, BC3, BC4, and BC5) from a 2-yr-old plantation consisting of 755 genotype samples were harvested together with primary coppice shoots from samples of PD (PD1-pedigree and PD2-pedigree), PT (PT1-pedigree) and first-generation poplar (F1) for physical, anatomical, chemical, and thermal analysis.

Anatomic, Physical, and Thermochemical Analysis

Anatomic analysis for each sample was conducted in cross, radial, and tangential sections with a scanning electron microscope (SEM; S-400; Hitachi, Tokyo, Japan). Thin wood sheets (0.2 mm thick from cross, radial, and tangential sections) were cut from each sample using a microtome. Dried sample sheets were glued on silicon wafers and then coated with gold via an ion sputter coater prior to observation by SEM. Operating voltages of SEM were 20 kV, and a magnification of 150 was used.

After samples were collected from the designated site, they were processed into small blocks for anatomic, moisture content, and specific gravity determination. Hot water extractives and acid-insoluble lignin and holocellulose contents in the various poplar woods were determined in accordance with ASTM D 1110-84 and 1106-96, respectively (ASTM 2001). A hot water extraction method usually gives a good estimate of wood extractives, especially for hardwoods such as poplars, which is more cost- and labor-effective.

Proximate Analysis

Content of volatile matter, carbon, and ash in wood was evaluated based on ASTM D3172 (ASTM 2006) and D1102-84 (ASTM 2001). One gram of each sample was placed in an oxygen bomb calorimeter (Parr 6300) for heating value determination. After running each sample through the bomb calorimeter, corrections were made based on calorimeter gross and net heat of combustion (Eqs 1 and 2) for hydrogen, nitric acid, sulfuric acid, and fuse wire consumption for the final computation of gross and net heating value (Parr Instrument Co 2005) following ASTM E711-87 (ASTM 2006). After completion of all physical, chemical, proximate, and thermal tests on wood samples, analysis of variance and a t-test ($\alpha = 0.05$) were performed using the Statistical Analysis System (SAS, Cary, NC) package.

$$H_c = \frac{W \times T - e_1 - e_2 - e_3}{m} \tag{1}$$

$$H_n = 1.8 \times H_c - 92.7 \times H \tag{2}$$

where H_c = gross heat combustion (J/kg); T = temperature change (%); W = energy equivalent of calorimeter being used (J/kg); e_1 = heat produced by burning the nitrogen portion of air trapped in the bomb to form nitric acid (J); e_2 = heat produced by formation of sulfuric acid from the reaction of sulfur diode, water, and oxygen (J); e_3 = heat produced by heating wire and cotton thread (J); m = mass of sample (kg); H_n = net heating value (J/kg); and H = percentage of hydrogen (%) (based on a typical value of 6.2%).

TGA-FTIR Analysis

Thermal degradation behavior and pyrolysis tests of selected samples were investigated by a thermogravimetric analyzer (TGA Q50; TA Instruments, New Castle, DE). Temperature ranged from 22-400°C with a heating rate of 20°C/min. Nitrogen gas, at a rate of 20 mL/min, was used to avoid oxidation. Sample weights were approximately 2-3 mg. Evolution of gases and light volatile organic compounds were measured with the TGA analyzer connected via a heated transfer line to a NicoletiS10 (Thermo-Scientific, Waltham, MA) Fourier Transform IR Spectrometer (FTIR). Spectra of the gas mixture were measured every 30 s at 4 cm^{-1} resolution. A 1-min delay between TGA and FTIR gas cells has been taken into account in data analysis and presentation.

RESULTS AND DISCUSSION

Scanning Electron Microscopy Anatomic Observation

In general, all poplar species examined presented diffuse porous structure and agreed with prior research findings (Panshin and de Zeeuw 1980; Balatinecz et al 2001). Based on our visual estimates, all anatomic features such as vessel size and vessel cell pattern located at cross, radial, and tangential sections appeared different among PT, PD, and hybrid poplar species (Figs 2-5). PD (Fig 3) appeared to have more vessels per unit area compared with other poplar species. Vessel distributions in cross-sections were visually

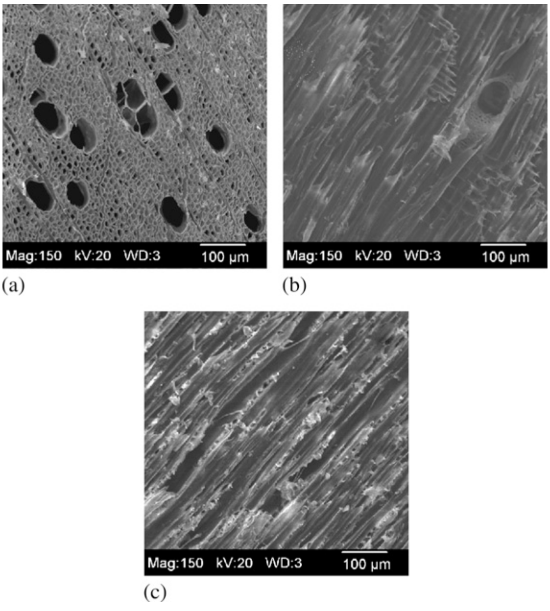

Figure 2. Scanning electron microscopy images of *Populus trichocarpa* (PT1): (a) cross-section, (b) radial section, and (c) tangential section.

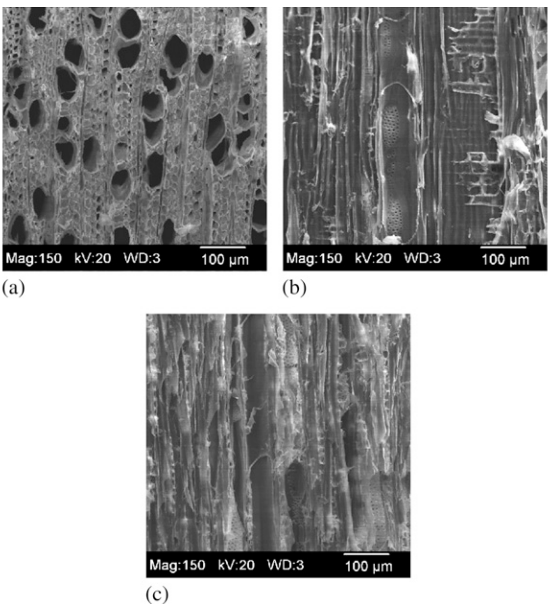

Figure 3. Scanning electron microscopy images of *Populus deltoides* (PD2): (a) cross-section, (b) radial section, and (c) tangential section.

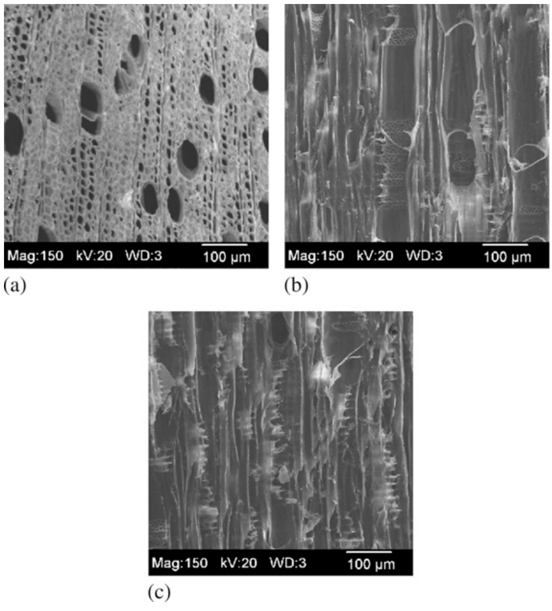

Figure 4. Scanning electron microscopy images of first-generation hybrid poplar (F1): (a) cross-section, (b) radial section, and (c) tangential section.

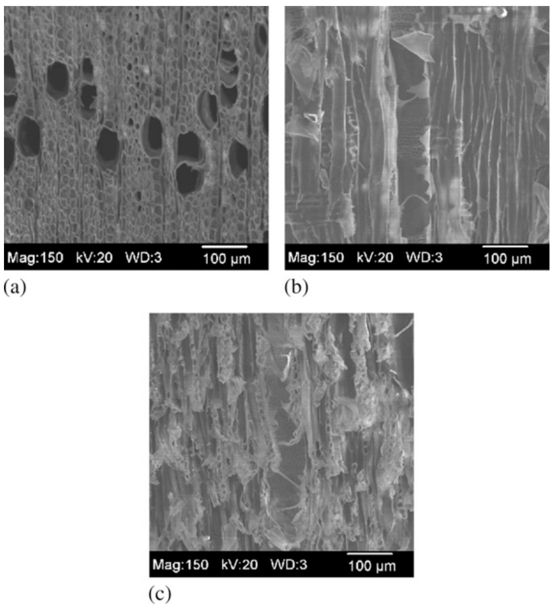

Figure 5. Scanning electron microscopy images of second-generation hybrid poplar (BC1): (a) cross-section, (b) radial section, and (c) tangential section.

different among poplar types. The anatomic result tended to suggest that hybrid poplars (Figs 4 and 5) with fewer vessels per unit area have more resemblance to PT (Fig 2) than to the pedigree father (PD clone) (Fig 3). Based on our ocular estimations, the predominant volumetric composition of observed poplar anatomy was fiber elements and vessel cells, which tends to agree with past research that poplar trees have more fiber proportion than vessels (Panshin and de Zeeuw 1980).

Physical and Thermochemical Analysis

Average specific gravity of the poplars showed some differences among PD, PT, and hybrid samples (Table 1). However, differences were not statistically significant ($p = 0.1431$). Poplar trees (including hybrid poplar) planted in North America have been shown to be diffused porous in structure with a low specific gravity ranging from 0.30 to 0.39 (Bendtsen et al 1981; Balatinecz et al 2001). However, average specific gravity observed in this study was slightly higher. Higher specific gravities of 0.44 and 0.42 were observed in stump samples of pedigree parents PT, PD, and hybrid poplar (F1, BC) species, respectively, whereas a lower specific gravity range of 0.35-0.39 was observed throughout the stem. Generally, specific gravity of BC1-BC5 was higher than other genotypes, and specific gravity of stump mass for most poplar species was slightly higher than those of their respective stems. This outcome was expected because stumps were larger in diameter and contained more fiber per unit area than stems. Higher specific gravity in the stump could be a result of higher fiber proportion because wood-specific gravity is significantly influenced by vessel-to-fiber ratio (Balatinecz et al 2001).

Content of measured chemical properties (extractives, lignin, and holocellulose) in poplar wood (Table 1) generally agreed with that obtained in previous studies (Baker 1983; White 1987; Adebola et al 2009). Extractive content ranged from 10.6-11.1% for PD1, PD2, and PT; 11.0-11.3% for F1 and BC1; and 8.8-9.5% for BC2-BC5. Extractive content of BC2-BC5 showed a statistically significant difference ($p \leq 0.0001$) from other poplar species tested. Lignin content among the major group of poplar trees was significantly different ($p = 0.04599$). PD1 had the highest average lignin content of 25.6% followed by F1 and BC1, which had average lignin contents of approximately 25%. Observed significant differences among poplar trees do not indicate a

Table 1. Physical and chemical properties of hybrid poplar (first generation, F1) and backcross (BC), *Populus deltoides* (PD1 and PD2), and *Populus trichocarpa* (PT1).

Poplar species	Wood location	Diameter (mm)	Specific gravity	Extractives (%)	Lignin (%)	Holocellulose (%)
PD1	Stem	14.5	0.35	10.71	25.60	63.29
	Stump	65.0	0.35	10.64	25.60	64.00
PD2	Stem	14.0	0.39	10.98	23.64	65.24
	Stump	31.0	0.44	10.98	23.64	65.24
PT1	Stem	19.0	0.36	11.11	23.38	65.51
	Stump	65.0	0.41	10.15	23.10	67.00
F1	Stem	16.0	0.39	11.09	25.00	63.91
	Stump	85.0	0.43	11.30	22.58	66.32
BC1	Stem	13.5	0.35	11.00	24.98	63.88
	Stump	31.0	0.43	11.35	24.67	63.44
BC2 (2-445)	Stem	25.4	0.42	9.00	24.00	68.00
	Stump	46.6	0.41	9.30	24.00	68.10
BC3 (1-425)	Stem	31.5	0.42	8.80	24.00	69.20
	Stump	50.0	0.43	9.20	25.00	66.80
BC4 (124)	Stem	31.5	0.42	8.80	24.20	69.20
	Stump	34.0	0.42	9.31	25.00	67.00
BC5 (031)	Stem	18.0	0.35	9.50	23.00	68.00
	Stump	40.0	0.41	9.00	23.00	69.00

useable difference with respect to bioconversion of wood to fermentable sugars, which contrasts with a previous report that wood enzymatic hydrolysis increases with decreased lignin content (Kim et al 2000). Average lignin content of the remaining genotypes ranged from 24.0-22.6%. The relatively higher average lignin content of hybrid poplars observed in this study agrees with previous findings that reported hybrid poplars had high lignin content (Labosky et al 1983; Law and Rioux 1997). However, average lignin content of hybrid poplar stem and its pedigree is 2-6% lower compared with yellow-poplar and red oak stem woods (Adebola et al 2009). The reason for lower average lignin content could be the age of poplar trees used in this study. There were statistically significant differences (p = 0.00026) in holocellulose content among various poplar trees investigated. Holocellulose content of stump biomass was 67.0, 66.3, 64.0, 65.2, and 63.4% for PT1, F1, PD1, PD2, and offspring BC1, respectively. Slightly higher holocellulose content (67.0-69.2%) was observed among offspring BC2-BC5. Compared with their parent species, the higher holocellulose content of hybrid poplar species indicated that hybrid poplar could be suitable for biofuel conversion through biochemical process.

Proximate Analysis

Proximate analysis indicated similar volatile matter, carbon, and ash content among various poplar trees (Table 2). Values obtained were within the range previously reported for hardwood species (Sjostrom 1981; Demirbas 1997; Adebolo et al 2009). Generally, observed stem/stump volatile matter content, which ranged from 72.0-74.7%, showed little variation within wood (stem vs stump). The F1 and PD2 genotypes exhibited a slightly higher volatile matter content of 74.7%. Stump volatile content of hybrid poplar (BC1) was slightly higher than PD2 at the expense of lower carbon content. PD1, PT1, and BC had high carbon content ranging from 27.2-28.0% compared with other poplar tree samples. The least carbon content of 25.2% was found in stem biomass of PD2. Ash content (ie minerals such as magnesium, calcium, potassium) varied significantly among genotypes. Stump ash content (0.8-1.7%) was higher than stem ash content (1.1-1.3%) in most genotypes. Hybrid poplar (F1 and BC1) showed slightly lower ash content compared with their parents (1.1-1.2% vs 0.8-1.7%).

Measured heating value varied significantly among poplar types. Values observed along the

Table 2. Proximate and heat-related properties of hybrid poplar (first generation, F1) and backcross (BC), *Populus deltoides* (PD1 and PD2), and *Populus trichocarpa* (PT1).

Poplar species	Wood location	Specific gravity	Volatile (%)	Carbon (%)	Ash (%)	Heat (kJ)
PD1	Stem	0.35	72.3	27.7	1.3	8326
	Stump	0.35	71.5	28.5	1.2	8046
PD2	Stem	0.39	74.7	25.2	1.2	8065
	Stump	0.44	73.6	26.4	1.7	7610
PT1	Stem	0.36	72.9	27.1	1.2	8221
	Stump	0.41	72.6	27.4	0.8	7506
F1	Stem	0.39	74.7	25.3	1.2	8322
	Stump	0.43	74.6	25.4	1.1	8039
BC1	Stem	0.35	72.4	27.2	1.1	8299
	Stump	0.43	73.3	27.2	1.2	8247
BC2 (2-445)	Stem	0.42	72.0	26.9	1.1	7874
	Stump	0.41	72.0	26.8	1.2	7545
BC3 (1-425)	Stem	0.42	73.0	26.0	1.0	8356
	Stump	0.43	72.0	26.0	1.3	8263
BC4 (124)	Stem	0.42	71.0	28.0	0.9	8263
	Stump	0.42	74.0	25.0	1.0	8356
BC5 (031)	Stem	0.35	73.0	26.0	1.1	8059
	Stump	0.41	73.0	26.0	1.1	7498

stem of poplar species indicated a slightly higher gross heat value than in the stump. The heat values ranged from 7874-8356 kJ in the stem and from 7498-8356 kJ in the stump for various poplar trees (Table 2). Hybrid poplars (F1, BC) presented slightly higher gross heating values than their pedigree parents. The reason for this could be the proportionately higher lignin content of poplar samples. Lignin has been previously reported to have a strong correlation to higher heating value of wood (Baker 1983; White 1987). The potential higher heating values of hybrid poplar species also suggest that these species could be suitable as energy crops.

TGA-FTIR Analysis

Heat-related properties of poplars were further examined with TGA at various pyrolysis temperatures (from 22-400°C). All poplar samples examined showed a similar pattern of degradation (Fig 6a). TGA graphs indicated two main phases in wood decomposition. Phase 1 showed equilibrium of moisture content loss at low temperature (<230°C). Degradation of cellulose and lignin into volatiles (synthetic gases such as CO_2, CH_4, CO, H_2O, and other chemicals) occurred in phase 2 at approximately 250°C (Fig 7). Significant degradation of wood cell walls and lignin of poplar trees, with respect to temperature, occurred between 250 and 390°C, as indicated in the thermogravimetric derivative curve (TGD) peaks (Fig 6b). TGD curves, however, showed obvious differences in degradation behavior between hybrid poplars and other poplar trees because pyrolysis behaviors of the three basic wood components, hemicellulose, cellulose, and lignin, were significantly different (Fig 6a) (Nowakowskia et al 2007; Yang et al 2007).

Hybrid poplar BC had lower residue amounts at 400°C, which was attributed to its lower lignin content compared with other genotypes, which had much higher residue at this temperature (Fig 6). TGD curves of hybrid poplar also showed some stability with decreased mass loss rate during phase 2 of degradation. The stability observed in this study could be a result of the slightly higher proportion of lignin in the hybrid poplars. A previous study (Sun et al 2000) indicated that lignin, in contrast to cellulose, degrades slowly with a high rate of nonvolatiles. Lignin's thermal stability is caused by its complex hydrocarbon structures of benzene–phenolic compounds (coumarylic, sinalpylic, and coniferylic) (Yang et al 2007).

Typical spectral outputs of hybrid poplar and its cellulose from TGA-FTIR analysis were plotted in the form of 3D spectra (Fig 7). FTIR spectra plots illustrated the evolving of gas products during pyrolysis of samples as a function of wave number and temperature. Temperatures at

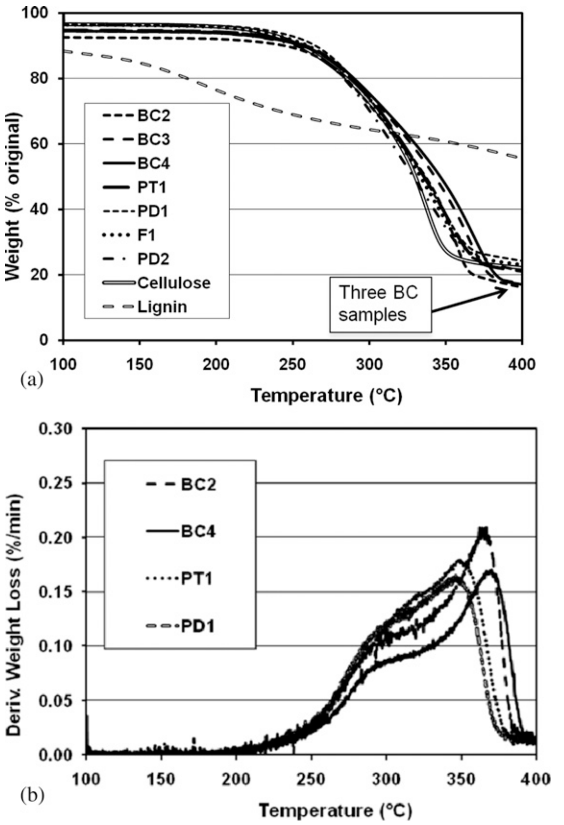

Figure 6. Thermal degradation: (a) weight loss of seven wood samples and cellulose and lignin from hybrid poplar; and (b) derivative weight loss of two hybrid samples (BC2 and BC4) and parent poplars (PD1 and PT1) at 0-400°C tested by TGA.

which gas products were released (Fig 7) corresponded to observed sample weight loss (Fig 6). The main volatile components identified by FTIR included CO_2, CH_4, CO, H_2O, and some organics (a mixture of acids, aldehydes [C=O], alkanes [C–C], and ethers [C–O–C]). However, release time and temperature of some gases were different in hybrid poplar compared with its cellulose because it is more difficult for lignin to decompose than cellulose and hemicellulose. Lignin in hybrid poplar particles consists of aromatic groups, which have higher chemical bond energies than polysaccharide structures of cellulose, which consist mostly of a series of glucose molecules (Yang et al 2007). For example, more CO_2 was released at higher temperature from hybrid poplar than from the cellulose sample (Fig 7).

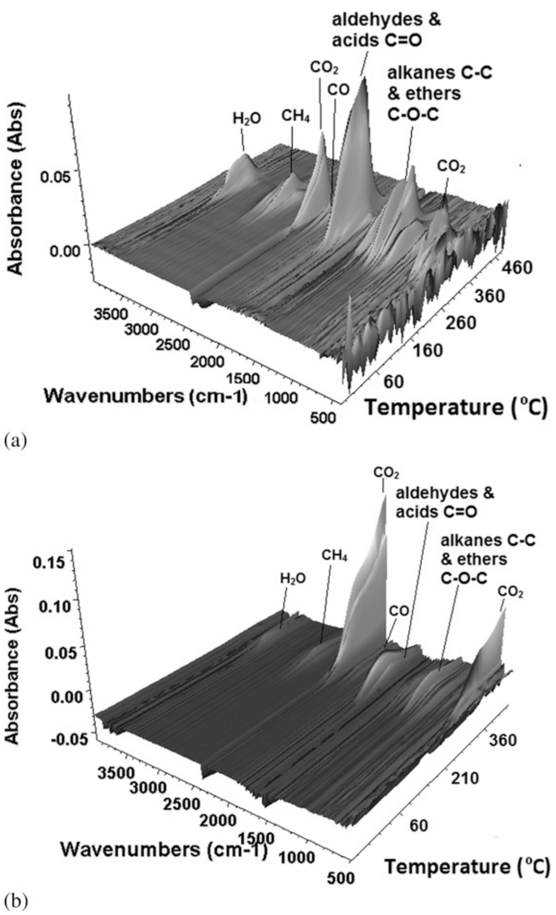

Figure 7. Typical Fourier Transform IR Spectrometer spectra of gas products: (a) from pyrolysis of hybrid poplar cellulose and (b) from hybrid poplar wood particles.

CONCLUSIONS

The cross between poplar species PD and PT resulted in hybrid poplar trees with different anatomic, physical, chemical, and thermal properties from their parent species. All observed poplar trees had different diffuse porous structure with distinct patterns of vessel distribution. Vessel distributions in cross-sections were visually different among poplar types. Average specific gravity of hybrid poplar was slightly higher than in previous findings. Extractive content was statistically significant among PD, PT, F1, and BC. Lignin content among the major group of poplar trees was significantly different. Holocellulose content of hybrid poplar species was generally higher than that of their parent species. Proximate analysis of volatile matter, carbon, and ash content showed no significant differences among the various poplar species. Heating values varied significantly among poplar species. Gross heating value of stem biomass of different poplar species was slightly higher than that of stump samples. Hybrid poplars (F1, BC) presented slightly higher gross heating values than their parent species. TGD curves indicated different patterns of degradation between hybrid poplars and their parents during phase 2 of thermal degradation. The main volatile components generated were CO_2, CH_4, CO, H_2O, and some organics identified by TGA-FTIR analysis. Hybrid poplars could be a suitable energy crop for biofuel production through either biological or thermochemical conversion.

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