Biomass Characterization of *Buddleja davidii*: A Potential Feedstock for Biofuel Production

BASSEM B. HALLAC,[†] POULOMI SANNIGRAHI,[†] YUNQIAO PU,[†] MICHAEL RAY,[§]
RICHARD J. MURPHY,[§] AND ARTHUR J. RAGAUSKAS*,[†,#]

Institute of Paper Science and Technology, School of Chemistry and Biochemistry, Georgia Institute
of Technology, Atlanta, Georgia 30332; Division of Biology, Imperial College London, London,
United Kingdom; and Forest Products and Chemical Engineering Department, Chalmers University of
Technology, Kemivägen 10, Gothenburg SE-412 96, Sweden

A compositional analysis was performed on *Buddleja davidii* to determine its general biomass characteristics and provide detailed analysis of the chemical structures of its cellulose and lignin using NMR. *B. davidii* is a new potential lignocellulosic bioresource for producing bioethanol because it has several attractive agroenergy features. The biomass composition of *B. davidii* is 30% lignin, 35% cellulose, and 34% hemicellulose. Solid-state CP/MAS ^{13}C NMR showed that 33% of the cellulose is para-crystalline and 41% is at inaccessible surfaces. Both quantitative ^{13}C and ^{31}P NMR were used to examine the structure of lignin. The lignin was determined to be guaiacyl and syringyl with an *h*:*g*:*s* ratio of 0:81:19.

KEYWORDS: *Buddleja davidii*; biomass characterization; lignin; cellulose; hemicellulose; NMR; GPC

INTRODUCTION

The demand for energy is increasing every year and is expected to grow by more than 50% by 2025, but the finite amount of fossil fuel available will be insufficient to satisfy this increase (*1, 2*). These demands and the recognition that society needs to develop sustainable energy technologies have focused the attention of many researchers on the development of alternative and renewable sources of energy, especially transportation fuels.

A promising sustainable source of energy is biofuels, including bioethanol from a variety of bioresources. Currently, bioethanol is produced primarily from the fermentation of sucrose and starches, which is frequently referred to as first-generation bioethanol (*2, 3*). In the United States and Brazil, this is accomplished using primarily corn starch and sugar cane, respectively (*1, 4*). The need to broaden this bioresource base has been acknowledged by many. Second-generation bioethanol provides an alternative technology that utilizes lignocellulosic materials such as wood agricultural or forest residues to produce biofuels. The benefits of this approach are numerous and include the facts that they (1) do not directly compete with food, (2) have a greater net energy generation per area of land, and (3) can be grown on nonagricultural lands.

Buddleja davidii Franch. is a shrub that originated in China, but has been naturalized in different parts of the world, including parts of the United States and Europe (*5*). This plant has several attractive agroenergy features that make it an interesting potential source for biofuels. *B. davidii* exhibits a very wide range of growth habitat and is well adapted to growing in many soil conditions (*6*). Moreover, the plant is perennial, has favorable growth dimensions (up to 3−5 m high), and has very few pests or diseases (*6*). This suggests *B. davidii* could be a valuable future agroenergy crop for second-generation ethanol production, although, to date, no attempts have been made to "domesticate" it as a crop species.

The process of producing bioethanol from lignocellulosic materials involves several steps (**Figure 1**) (*7*). Briefly, the material is pretreated to alter its structure so that the biomass matrix can be more accessible to enzymatic hydrolysis (*8*). During pretreatment, lignin is partially removed from the biomass, most of the hemicelluloses are often hydrolyzed, and the crystallinity of cellulose is disrupted to a certain degree. Enzymes, such as cellulases, can then be used to hydrolyze the cellulose to glucose, and glucose is then commonly fermented to ethanol (*7*).

Biomass characterization needs to be performed to understand the basic composition of the biomass and how its fundamental structure can be altered during pretreatment to optimize the
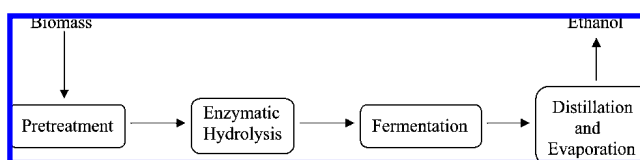

Figure 1. Process of a typical ethanol production scheme from biomass.

* Corresponding author [telephone (404) 894-9701; fax (404) 894-4778; e-mail arthur.ragauskas@chemistry.gatech.edu].
[†] Georgia Institute of Technology.
[§] Imperial College London.
[#] Chalmers University of Technology.

1276   *J. Agric. Food Chem.,* Vol. 57, No. 4, 2009

Hallac et al.

release of C6 and C5 sugars for fermentation. This study focuses on the determination of ash, extractives, lignin, hemicellulose, and cellulose content in *B. davidii*, as well as detailed elucidation of the chemical structures of both lignin and cellulose by NMR. The results will be used in future work to design the processes to convert *B. davidii* biomass into bioethanol.

MATERIALS AND METHODS

Sample Preparation. Several stems and foliar and floral parts of *B. davidii* were harvested from a single plant from a domestic garden in southern England in September 2007 and divided into three main constituents: leaves, bark, and stem. The samples were air-dried and prepared as follows: stems were manually debarked, chipped, and milled to pass a 0.40 mm screen; the leaves and bark were ground using a pestle and mortar. All of the results from the analyses were calculated on the basis of the oven dry weight of biomass that was determined by measuring the moisture content using a moisture analyzer. The analyses that include standard deviations were done in two replicates.

Ash and Extractive Contents. The extractives contents were measured according to TAPPI method T 204 cm-97, in which samples were extracted with dichloromethane (DCM), and the extractive content was calculated gravimetrically (*9*). Inductively coupled plasma (ICP) analysis was performed on all three samples for the analysis of trace inorganic elements (*10*). Finally, the ash content was measured by heating the samples at 525 °C in a furnace.

Tannin Content. DCM-extracted bark (2.00 g of dry weight) was Soxhlet extracted with a methanol/water mixture (75% v/v) for 24 h and then freeze-dried to recover the tannins (*11*).

Heating Value. The heating values of the leaves, bark, and stem were measured for combustion in an adiabatic oxygen bomb calorimeter according to TAPPI method T 684 om-06 (*12*).

GC-MS Analysis. The DCM extractives were concentrated under a stream of nitrogen at room temperature and then derivatized using *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA). The prepared sample was analyzed by employing GC-MS with splitless injection. The instrument used was a Hewlett-Packard 5890 II GC equipped with Hewlett-Packard 5971A mass selective detector. A 0.25 mm × 60 m DB-5 fused silica capillary column with a 25 μm coating stationary phase was used for the chromatographic separations. The GC conditions were as follows: initial temperature, 150 °C; initial time, 5 min; rate, 15 °C/min; final temperature, 280 °C; final time, 25 min; inject port temperature, 250 °C. The mass detector was operated by using the following conditions: EI model; 70 eV; filament on delay time, 8 min; mass scan range, 45−650 mu. Quantification of individual components was based on the total ion peak area. GC response factor of each individual compound was assumed to be one for all calculations.

Carbohydrate and Lignin Composition. Carbohydrate profiles and lignin (Klason and acid soluble) content in the stem, bark, and leaves were determined using the Laboratory Analytical Procedures (LAPs) provided by the National Renewable Energy Laboratory (NREL) (*13*). Uronic acid content was determined using the colorimetric technique described by Scott (*14*).

Holocellulose Pulping. The holocellulose pulping was conducted according to a modified literature method (*15, 16*). The DCM-extracted stem material (9.00 g of dry weight) was dispersed into 750 mL of deionized water. Then glacial acetic acid (6.00 mL) and sodium chlorite (6.00 g, 6.63×10^{-2} mol) were added to the mixture, which was poured in a plastic pouch and sealed. The mixture was heated in a shaking water bath at 70 °C for 2 h. After 2 h, another batch of glacial acetic acid and sodium chlorite were added, and the mixture was heated in the shaking water bath for an additional 2 h. Finally, the solid residue (holocellulose) was filtered out and washed thoroughly with deionized water. The whole process was repeated until the holocellulose turned white, and the percentage of Klason lignin was very low.

Cellulose Analysis. Cellulose was isolated by refluxing a holocellulose sample (2.00 g of dry weight) in 2.5 M HCl (200.00 mL) according to the literature method (*17*). The cellulose was collected by filtration and allowed to dry. The solid-state CP/MAS ^{13}C NMR experiment and the spectral fitting were performed as previously described by Pu et al. (*18*). The NMR experiment was performed on a Bruker Avance-400 spectrometer operating at 100.59 MHz for ^{13}C. The sample was packed in a 4 mm ZrO$_2$ rotor and spun at 8 kHz. The data were acquired with 8129 scans.

Hemicellulose Analysis. Hemicellulose was extracted from the holocellulose according to the method described by Jacobs and Dahlman (*19*). Briefly, the holocellulose was treated with potassium hydroxide, the cellulose was removed by filtration, and the hemicellulose was precipitated in an ethanolic solution containing 30% acetic acid.

Lignin Structural Analysis. The methods described by Guerra et al. and Holtman et al. were used to isolate and purify milled wood lignin (MWL) (*20, 21*). The elemental analysis (C, H, and O) was performed by Atlantic Microlab, Inc., Norcross, GA. Three NMR experiments were performed: distortionless enhancement by polarization transfer (DEPT), quantitative ^{13}C NMR, and ^{31}P NMR spectroscopy (*22–24*). They were recorded on a Bruker Avance-400 MHz NMR spectrometer. DEPT-135 was acquired using standard Bruker pulse program, 5 s pulse delay, and 8192 scans. Quantitative ^{13}C NMR spectrum was acquired using DMSO-d_6 (500 μL) as solvent for lignin (80 mg), with an inverse gated decoupling sequence, 90° pulse angle, 12 s pulse delay, and 12288 scans. For quantitative ^{31}P NMR, lignin was dissolved in a solution of pyridine/CDCl$_3$ and derivatized with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP), and the spectrum was acquired using an inverse gated decoupling pulse sequence, 90° pulse angle, 25 s pulse delay, and 200 scans. Cyclohexanol was used as an internal standard.

Gel Permeation Chromatography (GPC) Analysis of Cellulose. The number-average molecular weight ($\overline{M}_n$) and the weight-average molecular weight ($\overline{M}_w$) were determined by GPC after tricarbanilation of cellulose (*25*). Cellulose sample (15 mg) was dried over P$_2$O$_5$ under vacuum (762 mm of Hg) at 40 °C for 24 h and then was derivatized by adding anhydrous pyridine (4.00 mL) and phenyl isocyanate (0.50 mL, 4.62×10^{-3} mol). The sealed reaction flask was kept at 65 °C with stirring until the cellulose was completely dissolved. Afterward, the solution was cooled, and methanol (1.00 mL) was added to the reaction mixture to eliminate the unreacted phenyl isocyanate. The mixture was then poured into a 3:7 water/methanol mixture (100 mL), and the precipitated cellulose tricarbanilate was removed by centrifugation. The derivatized cellulose was purified by repeated washing with water/methanol (3 × 100 mL) followed by water (2 × 100 mL). Finally, the cellulose was freeze-dried and vaccum-dried for GPC analysis.

The cellulose tricarbanilate was analyzed for molecular weight and molecular weight distribution using a Hewlett-Packard 1090 series HPLC system consisting of an autosampler, a UV detector, and three columns of Styragel HR1, HR3, and HR4 (Waters Inc.) linked in series using tetrahydrofuran (THF) as the eluent. The derivatized cellulose was dissolved in THF (1 mg/mL), and the solution was filtered through a 0.45 μm filter. Then the filtered solution (20 μL) was injected into the HPLC system and was detected using the UV detector at 236 nm. Standard narrow polystyrene samples were used to construct a calibration curve. Data were collected with Agilent ChemStation rev. A.10.01 and analyzed with Agilent GPC Addon rev. A.02.02 software. The values $\overline{M}_n$ and $\overline{M}_w$ were calculated using the GPC software, and the weight-average degree of polymerization (DP$_w$) was obtained by dividing $\overline{M}_w$ by 519, the molecular weight of the cellulose tricarbanilate monomer.

GPC Analysis of Lignin. The $\overline{M}_n$ and $\overline{M}_w$ of isolated lignin were determined by GPC after acetylation of lignin to allow dissolution in THF (*26*). About 20 mg of lignin was dissolved in a 1:1 acetic anhydride/pyridine mixture (1.00 mL) and kept for 24 h at room temperature. Ethanol (25.00 mL) was added to the reaction mixture, left for 30 min, and then removed with a rotary evaporator. The addition and removal of ethanol was repeated seven times to ensure complete removal of acetic acid and pyridine from the sample. Afterward, the acetylated lignin was dissolved in chloroform (2.00 mL) and added dropwise to diethyl ether (100.00 mL) followed by centrifugation. The precipitate was washed three times with ether and then dried under vacuum (762 mm of Hg) at 40 °C for 24 h. GPC analysis for lignin was performed the same way as described for cellulose, except that the UV detector was set at 280 nm.

Table 1. Ash and Extractive Contents

	leaves	bark	stem
ash (%)	6.30 ± 0.07	4.75 ± 0.07	0.73 ± 0.06
extractives (%)	3.22 ± 0.07	2.83 ± 0.04	0.30 ± 0.01

Table 2. Inorganic Trace Elements Profile

	element[a]											
sample	K	Ca	P	S	Mg	Na	Fe	Si	Sr	B	Mn	Cd
leaves	7855	16050	2865	2260	853	143	117	83	48	41	43	0.2
bark	16400	5705	1850	790	1452	434	48	30	32	20	17	0.3
stem	2470	1175	541	268	174	128	14	9	7	6	4	0.2

[a] Element values are presented as mg of element/kg of sample.

Table 3. Summary of Heating Values

sample	heating value (MJ/kg)
leaves	19.77 ± 0.15
bark	21.31 ± 0.02
stem	20.32 ± 0.22

Table 4. Heating Values and Lignin and Extractive Contents of *Buddleja davidii* and Different Wood Species

wood species	Klason lignin (%)	extractives (%)	heating value (MJ/kg) unextracted wood	heating value (MJ/kg) extractive-free wood
B. davidii	30	0.30	20.32 ± 0.22	20.13 ± 0.05
softwood (27)				
Engelmann spruce	27	2.40	20.14	19.95
redwood	34	8.20	21.23	20.81
hardwoods (27)				
yellow poplar	21	3.80	19.65	19.46
basswood	18	6.70	20.00	19.30

RESULTS AND DISCUSSION

Ash and Extractive Analyses. The ash and extractive contents of the leaves, bark, and bark-free stems are summarized in **Table 1**. The leaves had a higher ash and extractive content than the bark and the stem because the leaves contain more inorganic elements as shown in **Table 2**. The ICP values have percent relative standard deviations (% RSD) ranging from 1 to 20%. The ICP analysis showed that the relative concentration of the elements in this plant was K > Ca > P > S > Mg > Na > Fe > Si > Sr > B > Mn > Cd. The distribution of the elements between the leaves, bark, and stem differed depending on the element, but as would be expected, the mineral elements were found predominantly in the metabolically active parts of the plant, that is, the leaves and bark. For subsequent bioprocessing of *B. davidii* into a biofuel, these nonprocessed elements represent a waste stream that will need to be addressed in an environmentally acceptable manner.

The heating values of the biomass constituents were measured to assess total energy values (**Table 3**). The heating value of *B. davidii* has been compared with other heating values from various wood species. **Table 4** shows that *B. davidii* has a heating value that compares favorably with those for softwoods and hardwoods. According to White (27), the heating value of wood is dependent on the amount of lignin and extractives; it increases with higher lignin and extractive contents. Overall, *B. davidii* stem wood has a heating value more akin to the higher values of softwoods than hardwoods, probably due to its relatively high lignin content at 30% on a dry weight basis.

One of the main operations of the future biorefinery, besides producing biofuels, would be to extract high-value chemicals that may already be present in or easily synthesized from the biomass (1). For instance, the tannin in the bark has been used for antioxidants (28). This plant has 9.45 ± 1.17% tannin in the bark. The foliage and flowers of *Buddleja* species are also known to contain anti-inflammatory compounds (flavonoids, carotenoids), antioxidant compounds (flavonoids and phenylethanoids), and antibacterial compounds (e.g., verbascoside against *Staphylococcus aureus*) (29, 30), which, although not investigated here, may also offer potentially high-value chemicals for extraction in biorefining. After the extraction of such high-value chemicals, the leaves and bark may be useful for combustion for heat and power production, composted for horticultural use, or investigated further as a potential animal feed (assuming protein, energy, and mineral contents are appropriate). They may also enter further processing for bioethanol production.

To identify other potential chemicals in *B. davidii*, GC-MS analysis was performed on the DCM extracts. The stem wood had a low content of extractives (**Tables 1** and **5**). However, the extractives content was approximately 10 times higher in the leaves and bark than in the stem. This was also reflected in substantially elevated levels of aromatics, alkanes, fatty acids, alcohols, and sterols in the leaves/bark than in the stem.

Biomass Composition. The cell wall macromolecular biomass composition of *B. davidii* is detailed in **Table 6**. *B. davidii* had a lower cellulose content and higher hemicellulose and lignin contents compared with some common softwood and hardwood species (28). The relatively high lignin content has potential implications for the effectiveness of pretreatment processes in releasing carbohydrates for fermentation, and the high hemicellulose content indicates a significant amount of C5 as well as C6 sugars available for conversion to bioethanol. The carbohydrate profile in **Table 7** indicates a relatively low glucose content (39%) for *B. davidii* stem material (28), compared with other wood species, and a glucose to xylose ratio of 1.8:1. Given the relatively high hemicellulose content, microorganisms capable of fermenting C6 and C5 sugars should appear to be desirable for the efficient conversion of this bioresource to ethanol. As expected for the bark, the lignin content was high (56%); the carbohydrate composition exhibited a few differences from the stem, but glucose and xylose were the dominant sugars present (respectively 17 and 11%) (**Table 7**). The leaves contained ~31% Klason lignin, no acid-soluble lignin, and low carbohydrate content with approximately 11% glucose.

Cellulose Analysis. The CP/MAS ^{13}C NMR spectrum and cellulose signal assignments are presented in **Figure 2**. The region in the NMR spectrum of cellulose between δ 80 and 92 is the most informative region, which is assigned to C-4 carbons (18). The area between δ 86 and 92 represents the crystalline and para-crystalline forms of cellulose, whereas the broader signal between δ 80 and 86 corresponds to the amorphous domains (18).

To determine the relative amounts of cellulose I$_\alpha$, cellulose I$_\beta$, para-crystalline cellulose, and celluloses at accessible and inaccessible surfaces, an analysis method based on a nonlinear least-squares fitting of CP/MAS ^{13}C NMR spectra was performed (18, 31). **Figure 3** shows the spectral fitting for the C-4 region of the CP/MAS ^{13}C NMR spectrum of cellulose, and the assignments of the signals are presented in **Table 8**. The cellulose crystallinity index was determined to be 0.55, which is high compared with that of aspen (0.47) and spruce

1278 *J. Agric. Food Chem.,* Vol. 57, No. 4, 2009

Hallac et al.

Table 5. Evaluation of DCM Extracts by GC-MS

compound	leaves	bark	stem
	sample[a]		
aromatic compounds	**13.49**	**171.97**	**4.28**
4-hydroxyphenylethanol	13.49	7.44	4.28
benzaldehyde		29.63	
vanillic acid		73.16	
α-hydroxy-α-(4-hydroxy-3-methoxy)phenylacetic acid		35.43	
isopimaradiene		13.50	
ferulic acid		12.81	
alkanes	**1000.76**	**143.80**	**0**
C23	51.90		
C25	56.78		
C27	88.92		
C29	594.25	107.93	
C31	208.91	35.87	
fatty acids	**1410.95**	**2583.43**	**187.12**
C14:COOH	28.72		
C16:COOH	365.24	275.52	95.20
C18:COOH	42.56	88.01	14.96
C19:COOH			2.20
C20:COOH	48.00	450.36	10.33
C21:COOH			2.79
C22:COOH	64.62	802.44	9.14
C23:COOH		12.51	5.23
C24:COOH	51.63	397.60	4.94
C26:COOH	17.47	35.97	
C28:COOH	18.92		
C30:COOH	28.95		
9,12-octadecadienoic acid	127.15	286.33	28.82
oleic acid		81.69	
linolenic acid	617.69	116.75	7.86
11-eicosaenoic acid		14.69	
2,3-dihydroxyhexadecanoic acid		21.56	3.33
azelaic acid			2.32
alcohols	**336.72**	**26.20**	**7.40**
C22:OH	25.46	15.52	5.99
C24:OH	232.29	10.68	
C28:OH	13.71		1.41
C30:OH	39.17		
C32:OH	26.09		
sterols	**159.41**	**99.74**	**69.91**
stigmasterol	36.66	52.37	26.14
sitosterol	122.75	47.37	41.60
ergosterol			2.17

[a] Compound values are presented as μg of compound/g of sample.

Table 6. Cell Wall Macromolecular Composition of *Buddleja davidii* and Other Wood Species

wood species	lignin (%)	cellulose (%)	hemicellulose (%)
	wood macromolecules[a]		
B. davidii	30	35	34
softwoods (*28*)			
Picea glauca	27	41	31
Pinus strobus	29	41	27
hardwoods (*28*)			
Populus tremuloides	21	48	27
Betula papyrifera	19	42	38

[a] All samples were analyzed extractive-free.

(0.45) (*32*). The highly crystalline structure of *B. davidii* cellulose suggests that there may be a need to tailor pretreatment technologies to decrease this value.

The values determined from the GPC analysis of cellulose were as follows: weight-average molecular weight ($\overline{M}_w$) = 5.21 × 10^5 g/mol; number-average molecular weight ($\overline{M}_n$) = 3.34 × 10^4 g/mol; polydispersity index ($\overline{M}_w/\overline{M}_n$) = 15.6; and weight-average degree of polymerization (DP$_w$) = 1004. The DP of cellulose in *B. davidii* is low compared to those of aspen (2500) and spruce (3300) (*33*). In contrast with the higher crystallinity index (see above), this lower DP$_w$ may be beneficial in the hydrolysis of cellulose by cellulase enzymes.

Structural Analysis of *B. davidii* MWL Using Quantitative **^{13}C NMR.** The MWL yield was 0.56% w/w of dry extractive-free wood. The elemental composition (% w/w dry MWL) was determined to be 60.48% C, 5.73% H, and 32.68% O, which resulted in chemical composition of $C_9H_{10.23}O_{3.65}$. The quantitative ^{13}C NMR spectrum is presented in **Figure 4**. This technique provides a facile method to determine the amount of various functional groups and lignin structures. A small peak of cinnamaldehyde was observed in the chemical shift of 194 ppm, which was also confirmed in the DEPT-135 spectrum. The region between δ 160 and 103 corresponds to the six aromatic carbons plus four vinyl carbons: two from the side chain of the cinnamaldehyde unit and two from the side chain of the cinnamyl alcohol unit. The integral value from δ 160 to 103 minus the integral values of vinyl carbons of both cinnamaldehyde and cinnamyl alcohol is set as the reference for quantifying the lignin structures as well as the functional groups. Therefore, the integral values for the structural moieties are reported per aryl group (*34*). **Table 9** summarizes the various lignin moieties, the chemical shifts, and the number/aryl group. The spectrum regions for *p*-hydroxyphenyl (*h*) units, aromatic quaternary C, and aromatic tertiary C have δ ranges of 162−157, 160−123, and 123−103, respectively. The DEPT-135 ^{13}C NMR spectrum confirms the chemical shifts for the CH$_3$, CH$_2$, and CH regions (**Figure 5**). No *p*-hydroxyphenyl lignin was detected by NMR. The guaiacyl-to-syringyl ratio (*g:s*) ratio was calculated on the basis of the number of carbons per aromatic ring in C-2 of guaiacyl unit and C-2/C-6 of syringyl unit (*35*). Because an uncondensed syringyl unit has two tertiary carbons (i.e., C-2/C-6), an approximate content of syringyl moieties/aryl group in the *B. davidii* MWL can be estimated from the total number of C-2/C-6 carbons divided by 2 (**Table 9**), that is, 0.18/aryl group ($I_{108-103}/2$). Because *h* units in the MWL are negligible as indicated by the quantitative ^{13}C NMR, the MWL consists of *g* and *s* with an approximate ratio of 81:19 (*g:s*). This ratio is low compared to those of other hardwood species such as birch (50:50) or *Eucalyptus grandis* (36:62) (*35*).

The degree of condensation (DC) in lignin is 45%, which is higher than that of loblolly pine (40%) (*36*) and *E. grandis* (21%) (*35*). According to Capanema et al., the DC can be determined by subtracting the experimental value of $C_{Ar−H}$ from the theoretical value of $C_{Ar−H}$ (*35*). The experimental value is the integral of the tertiary aromatic carbon region (δ 123.0−103.0), which is equal to 2.36 carbons/aromatic ring (**Table 9**), and the theoretical value (2.81 carbons/aromatic ring) is calculated from the following equation (*35*):

$$\text{theoretical } C_{Ar−H} = 2s + 3g + 2h \qquad (1)$$

Structural Analysis of *B. davidii* MWL Using Quantitative **^{31}P NMR.** This technique is based on the derivatization of phenolic structures with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) (**Figure 6**) (*24, 37*). The quantitative ^{31}P NMR spectrum for *B. davidii* MWL is presented in **Figure 7**. The concentration of each hydroxyl functional group was calculated on the basis of the internal standard and the integrated peak area (**Table 10**). The dominant OH

Table 7. Carbohydrate Profile and Lignin Content in *Buddleja davidii* Stem, Bark, and Leaves

| | relative carbohydrate composition[a] (mass %) | | | | | | lignin[b] (%) | |
	Ara	Gal	Glu	Xyl	Man	4-O-MeGlcA and GalA	Klason	acid-soluble
stem	0.48 ± 0.01	0.93 ± 0.02	38.88 ± 1.22	21.69 ± 0.65	3.04 ± 0.11	4.25 ± 0.21	29.68 ± 0.15	0.52 ± 0.01
bark	5.40 ± 0.31	2.45 ± 0.47	16.84 ± 2.30	11.06 ± 0.47	1.01 ± 0.47	2.69 ± 0.22	54.70 ± 0.09	1.09 ± 0.03
leaves	1.67 ± 0.31	1.64 ± 0.47	10.70 ± 2.30	4.04 ± 0.47	0.60 ± 0.47		30.57 ± 0.12	

[a] Mass sugar units/mass dry extractive-free wood. [b] Sample was analyzed extractive-free.
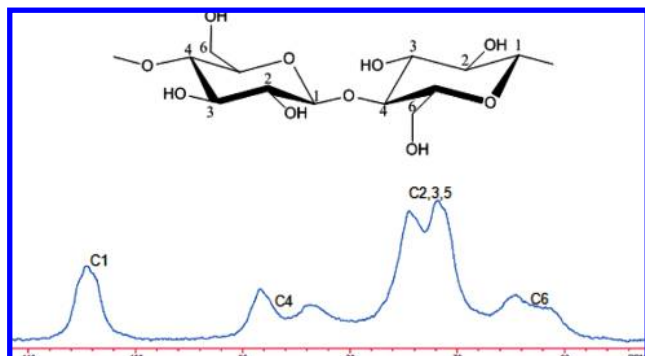
Figure 2. Solid-state CP/MAS ^{13}C NMR spectrum of cellulose.
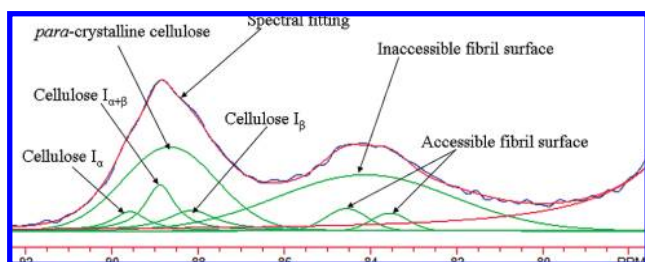
Figure 3. Spectral fitting for the C-4 region of the CP/MAS ^{13}C NMR spectrum of cellulose.

Table 8. Assignments of Signals in the C-4 Region of the CP/MAS ^{13}C NMR Spectrum

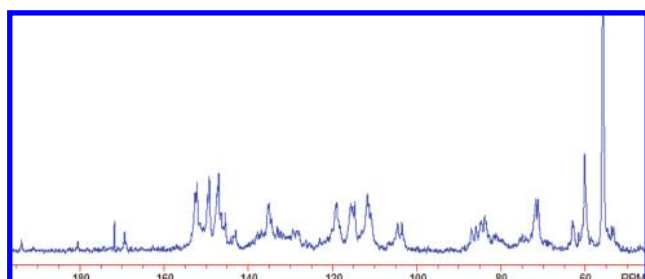| assignment	chemical shift (ppm)	FWHH[a] (Hz)	intensity (%)	line type
cellulose I_α	89.6	96	4.2	Lorentz
cellulose $I_{\alpha+\beta}$	88.9	85	8.7	Lorentz
para-crystalline cellulose	88.7	258	32.9	Gauss
cellulose I_β	88.2	142	6.5	Lorentz
accessible fibril surface	84.6	116	3.9	Gauss
inaccessible fibril surface	84.1	482	41.1	Gauss
accessible fibril surface	83.6	101	2.7	Gauss

[a] Full width at half-height.

Figure 4. Quantitative ^{13}C NMR spectrum of MWL from *B. davidii*.

group in the lignin is at aliphatic sites. The lignin has a considerable amount of free guaiacyl OH, but low carboxylic acid OH.

GPC Analysis of *B. davidii* MWL. The values determined from the GPC analysis were as follows: weight-average mo-

Table 9. Quantification of Several Lignin Moieties Using ^{13}C NMR

structure	δ ^{13}C NMR (ppm)	no./aryl group
γ-C=O in cinnamaldehyde	194.9–193.0	0.03
unconjugated carboxylic C=O	172.8–171.2	0.04
unconjugated carboxylic C=O	170.7–168.8	0.07
aromatic C—O bond: s-3,5[a], g-3,4[b]	160.0–140.0	2.20
aromatic C—O bond: s-4, g-4; aromatic C—C bond: C1	140.0–123.0	1.44
aromatic C—H bond: g-6	123.0–117.0	0.70
aromatic C—H bond: g-5	117.0–114.0	0.54
aromatic C—H bond: g-2	114.0–108.0	0.76
aromatic C—H: s-2,6	108.0–103.0	0.36
C_β in β-O-4; C_α in β-5 and β-β	90.0–78.0	0.97
C_α in β-O-4	78.0–67.0	0.93
$C\gamma$ in β-5, β-O-4 with α-C=O, and cinnamyl alcohol	65.0–61.3	0.25
$C\gamma$ in β-O-4 without α-C=O	61.3–58.0	0.56
methoxyl OCH_3	58.0–54.0	1.17
C_β in β-β and C_β in β-5	54.0–52.0	0.17

[a] Carbons 3 and 5 in syringyl lignin. [b] Carbons 3 and 4 in guaiacyl lignin.
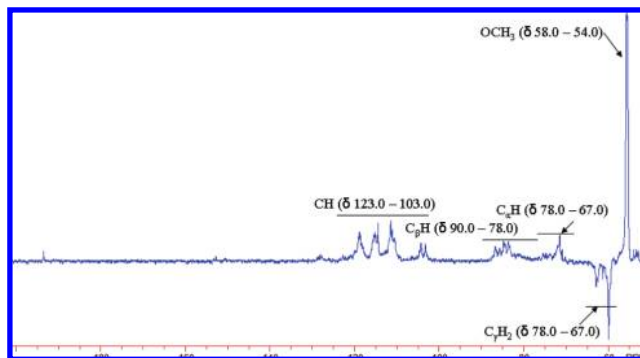

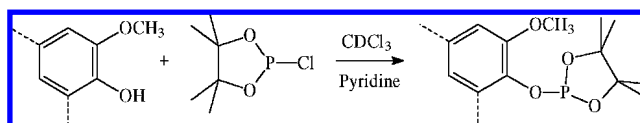**Figure 5.** DEPT-edited ^{13}C NMR spectrum of MWL from *B. davidii*.

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

lecular weight ($\overline{M}_w$) = 1.68×10^4 g/mol; number-average molecular weight ($\overline{M}_n$) = 7.26×10^3 g/mol; and polydispersity index ($\overline{M}_w/\overline{M}_n$) = 2.31. A value of 2.31 for the polydispersity index indicates a relatively large distribution of molecular weights in this lignin sample.

B. davidii has several unique features ranging from its distribution and growth habitat to aspects of its composition (analyzed here for the first time) that make it a potentially interesting bioresource for biofuels. The stem contains 30% lignin, 35% cellulose, and 34% hemicellulose. The principal hemicellulose is xylan, and the biomass has 22% xylose content based on oven-dried weight of extractive-free wood. It has a low cellulose DP$_w$ of 1000 compared with 2500–3500 for other wood celluloses. The predominant crystalline form of cellulose
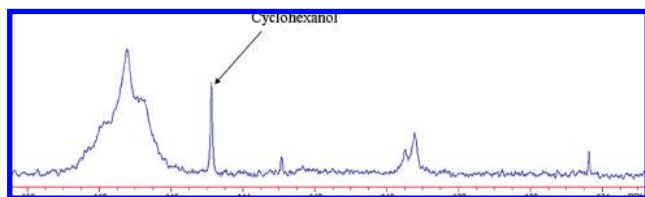
Hallac et al.

Figure 7. Quantitative ³¹P NMR of MWL from *B. davidii.*

Table 10. Quantification of Several Hydroxyl Functional Groups Using ³¹P NMR

structure	δ ³¹P NMR (ppm)	concentration (mmol/g of lignin)
aliphatic OH	150.0−145.5	4.51
condensed phenolic OH	144.7−140.2	0.27
guaiacyl	140.2−139.0	0.43
carboxylic acid OH	136.6−133.6	0.03

is para-crystalline cellulose (33%). Both guaiacyl and syringyl lignins are found in this plant with a *g:s* ratio of 81:19.

Although *B. davidii* has several "positive" features, it has some undesired characteristics such as relatively high lignin and hemicellulose contents, low cellulose content, and a high cellulose crystallinity index. Saccharification and fermentation yields and pretreatment evaluations will be needed to determine the balance of these positive and negative factors for assessing the biofuel production potential of *B. davidii* biomass.

ABBREVIATIONS USED

Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; 4-O-MeGlcA, 4-*O*-methylglucuronic acid; GalA, galacturonic acid.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Ragauskas, A. J.; Williams, C. K.; Davison, B. H.; Britovsek, G.; Cairney, J.; Eckert, C. A.; Frederick, W. J. Jr.; Hallett, J. P.; Leak, D. J.; Liotta, C. L.; Mielenz, J. R.; Murphy, R.; Templer, R.; Tschaplinski, T. The path forward for biofuels. *Science* **2006**, *311*, 484–489.

(2) Lange, J. P. Lignocellulose conversion: an introduction to chemistry, process and economics. *Biofuels, Bioprod. Biorefin.* **2007**, *1*, 39–48.

(3) Pu, Y.; Zhang, D.; Singh, P. M.; Ragauskas, A. J. The new forestry biofuels sector. *Biofuels, Bioprod. Biorefin.* **2007**, *2*, 58–73.

(4) Schubert, C. Can biofuels finally take center stage? *Nat. Biotechnol.* **2006**, *24*, 777–784.

(5) Findley, D. A.; Keever, G. J.; Chappelka, A. H.; Eakes, D. J.; Gilliam, C. H. Differential response of *Buddleia* (*Buddleia davidii* Franch.) to ozone. *Environ. Pollut.* **1997**, *98*, 105–111.

(6) Starr, F.; Starr, K.; Loope, L. *Buddleia davidii*: Butterfly Bush: Buddleiaceae. United States Geological Survey (Biological Resources Division): Hawaii, 2008; available at http://www.hear.org/pier/pdf/pohreports/buddleia_davidii.pdf.

(7) Galbe, M.; Zacchi, G. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv. Biochem. Eng./Biotechnol.* **2007**, *108*, 41–65.

(8) Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96*, 673–686.

(9) TAPPI Test Methods. Solvent extractives of wood and pulp, 2007.

(10) Allison, L.; Ragauskas, A. J. Metal profiling of southeastern US SW and HW wood furnish. *Tappi J.* **2000**, *83*, 97.

(11) Berg, A.; Navarrete, P.; Olave, L. Biochemicals and standardized solid fuels from *Radiata* pine bark. In *15th European Biomass Conference and Exhibition*, Berlin, Germany, 2007.

(12) TAPPI Test Methods. Gross heating value of black liquor, 2006.

(13) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J; Templeton, D.; Crocker, D. Determination of structural carbohydrates and lignin in biomass. *Laboratory Analytical Procedure*; National Renewable Energy Laboratory: Golden, CO. 2008.

(14) Scott, R. W. Colorimetric determination of hexuronic acids in plant materials. *Anal. Chem.* **1979**, *51*, 936–941.

(15) Vuorinen, T.; Alén, R. Carbohydrates. In *Analytical Methods in Wood Chemistry, Pulping, and Papermaking*; Sjöström, E., Alén, R., Eds.; Springer-Verlag: Berlin, Germany, 1998; pp 38−40.

(16) Zhang, D.; Pu, Y.; Chai, X.-S.; Naithani, V.; Jameel, H.; Ragauskas, A. J. Elucidating carboxylic acid profiles for extended oxygen delignification of high-kappa softwood kraft pulps. *Holzforschung* **2006**, *60*, 123–129.

(17) Liitiä, T.; Maunu, S. L.; Hortling, B.; Tamminen, T.; Pekkala, O.; Varhimo, A. Cellulose crystallinity and ordering of hemicelluloses in pine and birch pulps as revealed by solid-state NMR spectroscopic methods. *Cellulose* **2003**, *10*, 307–316.

(18) Pu, Y.; Ziemer, C.; Ragauskas, A. J. CP/MAS ¹³C NMR analysis of cellulase treated bleached softwood kraft pulp. *Carbohydr. Res.* **2006**, *341*, 591–597.

(19) Jacobs, A.; Dahlman, O. Characterization of the molar masses of hemicelluloses from wood and pulps employing size exclusion chromatography and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Biomacromolecules* **2001**, *2*, 894–905.

(20) Guerra, A.; Mendonca, R.; Ferraz, A.; Lu, F.; Ralph, J. Structural characterization of lignin during *Pinus taeda* wood treatment with *Ceriporiopsis subvermispora*. *Appl. Environ. Microbiol.* **2004**, *70*, 4073–4078.

(21) Holtman, K. M.; Chang, H.; Jameel, H.; Kadla, J. F. Quantitative ¹³C NMR characterization of milled wood lignins isolated by different milling techniques. *J. Wood Chem. Technol.* **2006**, *26*, 21–34.

(22) Pu, Y.; Ragauskas, A. J. Structural analysis of acetylated hardwood lignins and their photoyellowing properties. *Can. J. Chem.* **2005**, *83*, 2132–2139.

(23) Pu, Y.; Anderson, S.; Lucia, L.; Ragauskas, A. J. Investigation of the photooxidative chemistry of acetylated softwood lignin. *J. Photochem. Photobiol., A* **2004**, *163*, 215–221.

(24) Zawadzki, M. Quantitative Determination of Quinone Chromophore Changes during ECF Bleaching of Kraft Pulp. Ph.D thesis, Institute of Paper Science and Technology, 1999.

(25) Cohen, R.; Jenson, K. A. Jr.; Houtman, C. J.; Hammel, K. E. Significant levels of extracellular reactive oxygen species produced by brown rot basidiomycetes on cellulose. *FEBS Lett.* **2002**, *531*, 483–488.

(26) Lundquist, K. Proton (¹H) NMR spectroscopy. In *Methods in Lignin Chemistry*; Lin S. Y., Dence, C. W., Eds.; Springer-Verlag: Berlin, Germany, 1992; pp 242−249.

(27) White, R. H. Effect of lignin content and extractives on the higher heating value of wood. *Wood Fiber Sci.* **1986**, *19*, 446–452.

(28) Ragauskas, A. J.; Nagy, M.; Kim, D. H.; Eckert, C. A.; Hallett, J. P.; Liotta, C. L. From wood to fuels: integrating biofuels and pulp production. *Ind. Biotechnol.* **2006**, *2*, 55–65.

(29) Houghton, P. J.; Hylands, P. J.; Mensahb, A. Y.; Hensel, A.; Deters, A. M. In vitro tests and ethnopharmacological investigations: wound healing as an example. *J. Ethnopharmacol.* **2005**, *100*, 100–107.

(30) Avila, J. G.; de Liverant, J. G.; Martínez, A.; Martínez, G.; Munõz, J. L.; Arciniegas, A.; de Vivar, A. R. Mode of action of *Buddleja cordata* verbascoside against *Staphylococcus aureus*. *J. Ethnopharmacol* **1999**, *66*, 75–78.

(31) Wickholm, K.; Larsson, P. T.; Iverson, T. Assignment of noncrystalline forms in cellulose I by CP/MAS ¹³C NMR spectroscopy. *Carbohydr. Res.* **1998**, *312*, 123–129.

(32) Lennholm, H.; Larsson, T.; Iversen, T. Determination of cellulose I$_\alpha$ and I$_\beta$ in lignocellulosic materials. *Carbohydr. Res.* **1994**, *261*, 119–131.

(33) Coffey, D. G.; Bell, D. A.; Henderson, A. Cellulose and cellulose derivatives. In *Food Polysaccharides and Their Applications*; Stephen, A. M., Ed.; Dekker: New York, 1995; pp 125.

(34) Holtman, K. M.; Chang, H.-M.; Kadla, J. F. Solution-state nuclear magnetic resonance study of the similarities between milled wood lignin and cellulolytic enzyme lignin. *J. Agric. Food Chem.* **2004**, *52*, 720–726.

(35) Capanema, E. A.; Balakshin, M. Y.; Kadla, J. F. Quantitative characterization of a hardwood milled wood lignin by nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem.* **2005**, *53*, 9639–9649.

(36) Sannigrahi, P.; Ragauskas, A. J.; Miller, S. J. Effects of two-stage dilute acid pretreatment on the structure and composition of lignin and cellulose in loblolly pine. *Bioenerg. Res.* **2008**, *1*, 205−214.

(37) Granata, A.; Argyropoulos, D. S. 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a reagent for the accurate determination of the uncondensed and condensed phenolic moieties in lignins. *J. Agric. Food Chem.* **1995**, *43*, 1538–1544.