J For Res (2014) 19:233–237
DOI 10.1007/s10310-012-0386-6

SHORT COMMUNICATION

Quantifying lignin and holocellulose content in coniferous decayed wood using near-infrared reflectance spectroscopy

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Received: 19 July 2012 / Accepted: 15 November 2012 / Published online: 15 December 2012

Abstract To determine the independent decomposition rates of lignin and cellulose of decayed woody debris, a technique for the rapid analysis of lignin and cellulose is required. We applied a near-infrared spectroscopy (NIRS) technique to measure the lignin and holocellulose content in decayed wood. We succeeded in creating partial least-squares (PLS) models to estimate the lignin and holocellulose content in the decayed wood of five species using NIR spectra. Although the accuracy was acceptable for the estimation of a five-species mixed model ($R^2 = 0.970$ for lignin and $R^2 = 0.962$ for holocellulose), it was further improved when the model was applied to each species independently. This combination of NIRS and a PLS model is a valuable tool for the determination of the lignin and holocellulose content in decayed wood. The technique is time efficient (3 min per sample) and non-hazardous (no acid treatment is required).

Keywords Holocellulose · Lignin · Near-infrared spectroscopy · Partial least squares (PLS) regression

Introduction

The amount of woody debris in Japanese forest floors needs to be determined for the national inventory report under the United Nations Framework Convention on Climate Change; however, the amount of the decayed woody debris has not been investigated at the national scale. Recently (from 2011), the Japan Forest Agency began a systematic survey of dead wood biomass; however, the decay processes for woody debris on Japanese forest floors are poorly understood.

Because wood lignin is more resistant to decay than cellulose (Means et al. 1985; Ganjegunte et al. 2004; Butler et al. 2007), a precise decomposition model of wood decay process is required to understand the decomposition rate of lignin and cellulose independently. However, data regarding the lignin and cellulose content in decayed woods on the forest floor are lacking. One reason for this is that the analytical procedure for determining lignin and cellulose content is time consuming (currently 1 week is required for 24 samples in a typical laboratory) and complex, which limits the number of measurements that can be made. The analysis also requires acid digestion to decompose the holocellulose. Thus, improved analytic techniques are crucial for progress in this field of study. A novel method enabling a more time-saving measurement would contribute to an enlarged library of lignin and holocellulose content values for decayed wood and provide a more precise model to estimate woody-debris decomposition.

Recently, near-infrared spectroscopy (NIRS) has been applied for quantitative and qualitative analysis in various fields, e.g., soil carbon and nitrogen content (Brunet et al. 2007), various soil properties (Zornoza et al. 2008), and lignin and cellulose content in straw samples (Krongtaew et al. 2010a, b) and leaf-litter-layer samples (Ono et al. 2003, 2007, 2008). NIRS measures the spectral absorbance in the spectral regions from near-infrared to mid-infrared. When the specific absorbance bands of target chemicals are included in the observed spectral region, we can estimate

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the concentration or balance of chemicals by using spectral data and suitable regression model such as partial least-squares (PLS). Therefore, a combination of NIRS and PLS regression model can provide a more time-efficient and non-hazardous measurement technique for lignin and holocellulose content in decayed wood. In this report, we present results of the application of NIRS to measure the lignin and holocellulose content of decayed wood. This study is the first application of NIRS to determine the lignin and holocellulose content for Japanese decayed wood on the forest floors.

Materials and methods

Preparation of decayed wood

Samples of decayed wood from five dominant coniferous species in Japan, *Cryptomeria japonica* (Japanese cedar), *Chamaecyparis obtuse* (Japanese cypress), *Larix kaempferi* (Japanese larch), *Picea glehnii* (Glehn's spruce), and *Abies sachalinensis* (Sakhalin fir), were collected from 186 forests in 15 prefectures across the Japanese Archipelago. The sampling sites and methods have previously been described in detail (Sakai et al. 2011). The year since death ranged from 0 to 20 years. The bulk density of the wood ranged from 0.05 to 0.50 Mg m^{-3}. All samples were dried at 70 °C for at least 2 weeks. The bark was removed, and the residual wood was coarsely ground using a grinding mill, followed by fine grinding using a rotor mill (<0.50 mm; P-14, Fritsch). We collected 5–46 samples from each prefecture as standards to perform the calibration and validation of regression models. The total numbers of samples of each species collected for the calibration standards were Japanese cedar ($n = 69$), Japanese cypress ($n = 76$), Japanese larch ($n = 58$), Glehn's spruce ($n = 7$), and Sakhalin fir ($n = 8$).

Chemical component analysis

The holocellulose and lignin contents were determined by the Klason method (TAPPI 1997, 1998; Ono et al. 2007). Lignin content was defined as the sum of H_2SO_4-insoluble and H_2SO_4-soluble lignin. The H_2SO_4-insoluble lignin was corrected by subtraction from the ash content gravimetrically determined by dry combustion at 600 °C. The H_2SO_4-soluble lignin (AL, %) was determined by absorbance of 210 nm (Abs) using the following equation:

$$\mathrm{AL} = V \times \mathrm{Abs} \times 100/a/W,$$

where V is the volume of the solution (m^3), W is the sample weight (kg), and a is the weight absorbance coefficient (113 for cm path length, $m^3\ kg^{-1}\ cm^{-1}$).

The percentage of each fraction was calculated as the ratio of fraction weight to the original sample weight.

FT-NIRS measurements

The powder samples were oven-dried at 70 °C in 20-mL glass vials (2 cm in diameter) and stored in an electric desiccator prior to NIRS to minimize the interference effects of water. The sample thickness in the vials was more than 2 cm. FT-NIRS was performed on an FT-IR spectrometer (Nicolet 6700; Thermo Fisher Scientific) with an application for near-infrared reflectance measurement (Smart UpDRIFT; Thermo Fisher Scientific). After the sample vial was placed on the Smart UpDRIFT port, 100 scans between 10,000 and 4,000 cm^{-1} were collected at a spectral resolution of 8 cm^{-1} per sample. The average of three measurements was recorded. It took about 3 min per sample to measure.

Estimation of lignin and holocellulose content

The models for the estimation of lignin and holocellulose content were made by PLS regression using chemometrics software (TQ Analyst v.7; Thermo Electron). The spectral regions between 6,800 and 5,800 cm^{-1} and between 5,000 and 4,050 cm^{-1} were used for the model to minimize the interference of water vapor (approximately 5,300–5,100 cm^{-1}) and relatively low S/N ratio regions (>6,800 cm^{-1}). The second derivatives of the 1/log$R$ spectra were calculated with a Savitzky and Goray algorithm, applying a 19-point third-order polynomial smoothing filter. The PLS model was calibrated with the second derivatives of the spectra and the results of the chemical analysis. The model calibration was validated by the root mean square error of calibration (RMSEC) using randomly selected validation data. The robustness of the model was evaluated by the root mean square error of cross validation (RMSECV) using the leave-one-out method. To find clusters in the samples, we used the score plot of the first principal component (PC1) and the second principal component (PC2) of the PLS model. If the score plot scattered separately by tree species, it would be better to make each model for each species.

Results and discussion

Chemical component analysis

The range of lignin and holocellulose contents was 25.6–40.6 and 55.0–71.8 %, respectively (Table 1). The H_2SO_4-soluble lignin content ranged from 0.4 to 3.1 %, which was a relatively minor component. The average

Table 1 The range and average of each fraction of the standard (%)

Tree species	Lignin			Holocellulose		
	Min	Max	Average	Min	Max	Average
Cryptomeria japonica (Japanese cedar)	30.2	40.6	33.5	55.0	68.1	63.5
Chamaecyparis obtusa (Japanese cypress)	28.4	38.1	31.4	57.7	69.0	65.3
Larix kaempferi (Japanese larch)	26.6	34.5	29.7	62.3	70.9	67.6
Picea glehnii (Glehn's spruce)	25.6	34.6	28.6	62.6	71.8	68.2
Abies sachalinensis (Sakhalin fir)	26.4	34.1	29.5	61.8	69.5	66.9

lignin content of Japanese cedar (33.5 %) was greater than those of the other tree species. The average holocellulose contents of Japanese cedar and Japanese cypress (63.5 and 65.3 %, respectively) were lower than those of the other tree species.

NIR spectra

An example of the second derivative of the NIR spectra was shown in Fig. 1. For example, the height of the negative peak around 5,980 cm^{-1}, which indicated the absorption band of lignin (Fackler et al. 2007), correlated with the lignin content in the sample.

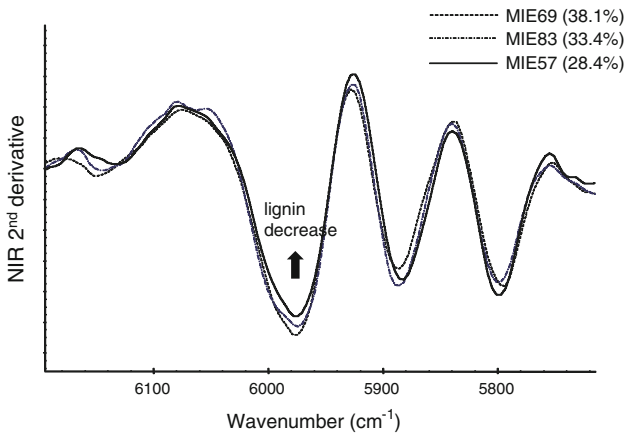

Fig. 1 An example of second derivatives of NIR spectra in the lignin dominated-region. The percentage in the parenthesis followed by the sample name in the line legend is the % lignin content

PLS regression model

PLS model using the data of all tree species

The coefficients of determination of the PLS model for lignin and holocellulose were both more than 0.95 when the data of all tree species were used for the model, indicating that the PLS model was appropriate for the estimation for the lignin and holocellulose content of these five tree species (Fig. 2; Table 2). The RMSEC value was less than 0.6 % for both lignin and holocellulose estimations, which was small relative to the average contents (approximately 30 and 70 %, respectively), also indicating the satisfactory performance of the model. The low RMSECV (less than 0.8 %) indicated that the model was robust enough for lignin and holocellulose estimation. There was no prominent higher value in the root mean square of errors for these models for each species (ranging from 0.35 to 0.58; Table 3), indicating that the models do not have an obvious bias by the tree species.

According to the PC1-PC2 score plot of the model for all species (Fig. 3), the PC2 value for Japanese cedar was greater than those of the other species, whereas the PC1 value for Japanese cypress was greater than those of the other species. From this result, we suggest that a better estimate might be achieved by using an individual PLS model for each species.

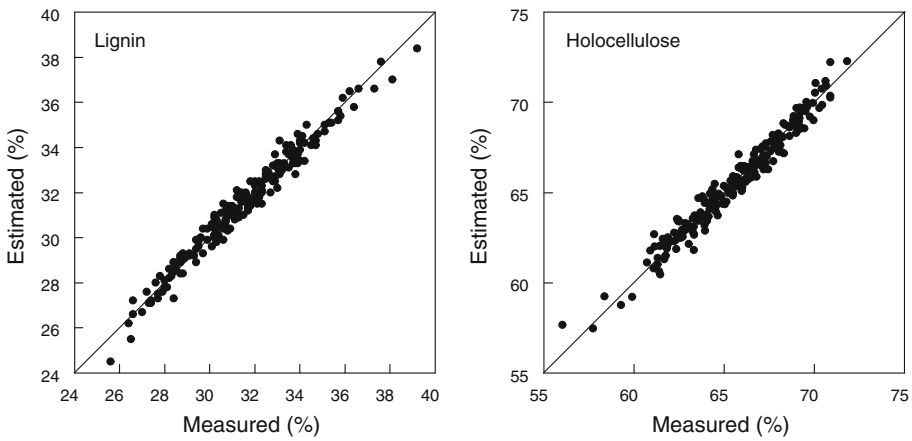

Fig. 2 NIRS-estimated and measured values of the PLS regression models for lignin (*left*) and holocellulose (*right*) for all species

Table 2 Parameters of partial least-square regression models of lignin and holocellulose

Species	Component	Number of samples	R^2	RMSEC	RMSECV
All species	Lignin	208	0.970	0.419	0.506
	Holocellulose	208	0.962	0.548	0.621
Cryptomeria japonica (Japanese cedar)	Lignin	69	0.977	0.294	0.515
	Holocellulose	69	0.982	0.325	0.592
Chamaecyparis obtusa (Japanese cypress)	Lignin	76	0.961	0.358	0.645
	Holocellulose	76	0.953	0.496	0.753
Larix kaempferi (Japanese larch)	Lignin	58	0.957	0.378	0.465
	Holocellulose	58	0.972	0.378	0.502
Picea glehnii + *Abies sachalinensis* (Glehn's spruce + Sakhalin fir)	Lignin	15	0.999	0.094	0.539
	Holocellulose	15	0.999	0.107	0.563

Table 3 Comparison of root mean square errors (RMSE) of each tree species in two models

Species	Component	All species model	Species specific model
Cryptomeria japonica (Japanese cedar)	Lignin	0.35	0.10
	Holocellulose	0.49	0.10
Chamaecyparis obtusa (Japanese cypress)	Lignin	0.47	0.38
	Holocellulose	0.58	0.38
Larix kaempferi (Japanese larch)	Lignin	0.38	0.35
	Holocellulose	0.56	0.48
Picea glehnii + *Abies sachalinensis* (Glehn's spruce + Sakhalin fir)	Lignin	0.50	0.28
	Holocellulose	0.57	0.32

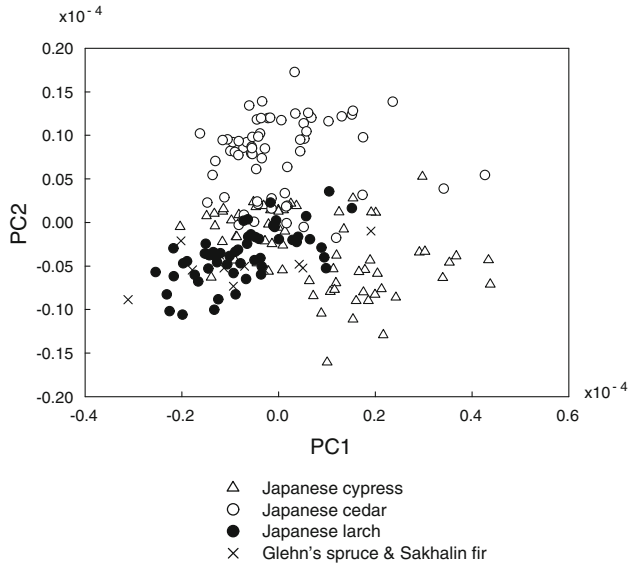

Fig. 3 The score plot of PC1 and PC2 of the PLS regression model for all species

The PLS model using the data of each tree species

The accuracy of the estimation of the PLS model was improved when it was applied to each species independently. For example, the RMSEC value decreased from 0.419 to 0.294 (Japanese cedar), 0.358 (Japanese cypress), 0.378 (Japanese larch), and 0.094 (Glehn's spruce + Sakhalin fir), respectively, for the estimation of lignin content (Table 2). The smaller root mean square of errors of each species-specific model relative to that of the PLS model for all species (Table 3) also indicates that the accuracy was improved in the species-specific model.

We successfully used PLS models to estimate the lignin and holocellulose content in the decayed wood of five species using spectra from NIRS. This combination of NIRS and a PLS model is a time efficient (3 min per sample) and non-hazardous (no acid treatment is required) tool for the determination of the lignin and holocellulose content in decayed wood.

As a general remark, because the condition and mechanical constituents of the NIR may be different from that in this study, the users of this method have to make a new PLS model for their NIR system using a set of calibration standards which have been identified beforehand in lignin and holocellulose content by the chemical component analysis (of course, our sample set is available for this purpose). The PLS model is only adaptable for the sample of which the lignin or holocellulose content was within the range of calibration standards. Therefore, if one would like to measure more decomposed samples which likely contain lignin or holocellulose out of the range of the calibration

set, they should develop the model to include these samples for the calibration standards after the lignin and holocellulose content have been measured by the chemical component analysis.

Acknowledgments We thank Drs. S. Kubo and T. Yamada for their assistance with FT-NIRS. We also thank Drs. K. Ono, A. Kataoka, and A. Sugimoto for their valuable input and M. Sanada, E. Sanada, R. Takeuchi, K. Misawa, A. Honda, C. Ogasawara, N. Kiuchi, Y. Okazaki, and Y. Sakamoto for their laboratory and computing assistance. This research was supported by a Grant-in-Aid for Scientific Research (B) 20380095 from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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