ELSEVIER

Bioresource Technology 74 (2000) 201–212

BIORESOURCE TECHNOLOGY

Estimating the chemical composition of biodegraded pine and eucalyptus wood by DRIFT spectroscopy and multivariate analysis

André Ferraz [a,*], Jaime Baeza [b], Jaime Rodriguez [b], Juanita Freer [b]

[a] *Departamento de Biotecnologia, Faculdade de Engenharia Quimica de Lorena, CP 116, 12600-000 Lorena, SP, Brazil*
[b] *Renewable Resource Laboratory, Universidad de Concepcion, Casilla 3-C, Concepcion, Chile*

Received in revised form 21 January 2000; accepted 23 January 2000

Abstract

Fourier transformed infra-red (FTIR) was evaluated as an analytical tool for monitoring wood biodegradation. A sample set containing typical soft (*Pinus radiata*) and hardwood (*Eucalyptus globulus*) decayed by six white- and two brown-rot fungi was prepared. Biodegradation times from 30 days to 1 year provided samples that suffered weight losses varying from 0.4% to 36% for pine wood and 1.7% to 42% for eucalyptus wood. Decayed samples were characterized by conventional wet chemical analysis and by diffuse reflectance FTIR (DRIFT) spectroscopy. Multivariate analysis was applied to correlate chemical composition in wood samples with the FTIR spectral data. Partial least squares (PLS) models were able to predict the major wood components' concentrations at the 99% confidence level presenting r^2 values higher than 0.86 in most cases. Models for *P. radiata* were more precise than for *E. globulus*.

Keywords: Wood biodegradation; FTIR; Multivariate analysis; *Pinus radiata*; *Eucalyptus globulus*; White-rot fungi; Brown-rot fungi

1. Introduction

The development of the biopulping technologies (Akhtar et al., 1998; Chen and Schimidt, 1995; Ferraz et al., 1996, 1998a; Messner and Srebotnik, 1994 and references therein), has made the selection of fungal species for lignin degradation a frequent subject of experimental research (Bettucci et al., 1992; Blanchette et al., 1992, 1987; Enoki et al., 1988; Ferraz et al., 1998b; Rodriguez et al., 1997; Setliff et al., 1990). However, the number of fungal species that can be assayed is limited because the characterization of decayed wood samples by wet chemical analysis is an arduous procedure.

The characterization of wood samples is a complex procedure involving several steps wherein wood components are isolated or degraded to monomeric fragments. These procedures destroy the wood matrix and require large sample sizes and long analysis time (ASTM D 1106-56; Dence, 1992; Ferraz et al., 1991; Irick et al., 1988; Kaar and Brink 1991; Kaar et al., 1991).

Fourier transformed infra-red (FTIR) spectroscopy has been used as a simple technique for analyzing wood samples or for monitoring structural wood changes during chemical or physical processing (Michell, 1988; Owen and Thomas, 1989; Michell, 1994). Contrary to wet chemical analysis, this technique requires small sample sizes and short analysis time. However, wood is a complex material composed of major macromolecules, namely, cellulose, polyoses, lignin and extractives. Owing to the complex nature of wood, most of FTIR observed bands cannot be directly assigned to one single component, and interpretation of isolated bands in wood FTIR spectrum can be misleading (Michell, 1988; Owen and Thomas, 1989). For this reason, multivariate analyses have been frequently used to correlate IR and also Near infra-red (NIR) spectral data with changes in structure or wood composition during chemical or physical processing. Based on these techniques several papers have reported rapid methods for estimating the concentrations of wood components (Michell, 1994; Olsson et al., 1995; Schultz et al., 1985). In all cases it is necessary to build up a representative group of samples and use it as a calibration data set.

Monitoring wood biodegradation by IR spectroscopy has been little studied. Usually, significant differences in wood spectra of decayed samples can be visualized only if wood is extensively degraded. Most of the published papers have focused on analysis of intensity changes of

* Corresponding author. Fax: +55-12-553-3165.
E-mail address: aferraz@debiq.faenquil.br (A. Ferraz).

0960-8524/00/$ - see front matter
PII: S0960-8524(00)00024-9

individual IR bands during wood biotreatment (Tseng et al., 1996; Hortling et al., 1992; Perez et al., 1993; Reeves III, 1993). This procedure is useful to provide results that corroborate those obtained through other wood characterization techniques. However, the use of FTIR spectroscopy for estimating wood component concentrations in biodegraded samples has not been attempted.

This paper focuses on the evaluation of FTIR as an analytical tool for monitoring wood biodegradation. A calibration set containing typical soft and hardwoods decayed by a variety of white- and brown-rot fungi was prepared. Biodegradation times from 30 days to 1 year were used to provide all stages of wood degradation in the calibration set. Decayed samples were then characterized by conventional wet chemical analysis and by diffuse reflectance FTIR (DRIFT) spectroscopy. Multivariate analysis was applied to correlate chemical composition in wood samples with the FTIR spectral data.

2. Methods

2.1. Wood preparation

Freshly cut chips (approximately $2.5 \times 1.5 \times 0.2$ cm) of 25-year old trees of *Pinus radiata* D. Don and 20-year old trees of *Eucalyptus globulus* were kindly furnished by Chilean Pulp and Paper Mills. Wet chips were air-dried and stored under dry conditions until used. Before incubation, 50 g of wood chips were immersed in water for 12 h. Afterwards the water was drained and the humid chips were sterilized at 121°C for 15 min. Sterilized wood chips were cooled and used in the biodegradation experiments (Ferraz et al., 1996).

2.2. Fungi and inoculum preparation

The white-rot fungi used in this work were: *Merulius tremellosus* ATCC 48745, *Poria medula-panis* ATCC 42463, *Trametes versicolor* (locally isolated), *Punctularia artropurpurascens* and *Ceriporiopsis subvermispora* (kindly furnished by Prof. M. Speranza from Universidad de la Republica de Montevideo, UY) and *Ganoderma aplanatum* (kindly furnished by Prof. E. Agosin from Universidad de Chile, CL). The brown-rot fungi were: *Poria cocos* ATCC 62778 and *Laetiporus sulfureus* ATCC 52600.

The fungi were grown on 200 ml of 2% malt-extract-solid-medium in 2 l Erlenmeyer flasks for 7–10 days. After fungal growth on the medium surface, 50 g of sterilized wood chips was added to the cultures and hand shaken for homogenization (Ferraz et al., 1996). Cultures were maintained stationary at 27°C for periods between 30 days and 1 year (prepared samples are presented in Tables 1 and 2). Growth was stopped after defined periods by washing all mycelium from the surface of the chips. Decayed chips were air-dried at 27°C and weighed. After weighing, the wood chip humidities were determined. Initial and final dry weights were used to determine weight losses. Weight loss owing to the sterilization procedure (1.6%) was subtracted from each weight loss value of biodegraded samples. Duplicate cultures for each biodegradation period were performed. A set of uninoculated sterilized wood chips served as a control wood sample.

2.3. Sampling

Decayed wood chips from culture duplicates were combined after weight loss determinations. Combined wood chips were milled in a knife mill (Manesco & Ranieri) to pass through a 0.5 mm screen. Approximately 3 g of milled sample was extracted with 95% ethanol for 6 h in a Soxhlet apparatus. Two extracted subsamples were taken for wet chemical analysis. For FTIR analysis, milled samples were sieved to pass through a 100 μm screen. Sieved samples were also divided into two subsamples for spectroscopic characterization.

2.4. Chemical analysis of wood samples

Extracted wood samples were hydrolyzed with 72% sulfuric acid at 30°C for 1 h (300 mg of sample and 3 ml of sulfuric acid). The acid was diluted to a final concentration of 3% (addition of 79 ml of water) and the mixture heated at 125°C/1 atm for 1 h. The residual material was cooled and filtered through porous glass filter number 3. The solids were dried to constant weight at 105°C and determined as insoluble lignin. The soluble lignin concentration in the filtrate was determined by the measurement of the absorbance at 205 nm and using the value of 105 $1\,g^{-1}\,cm^{-1}$ as the absorptivity of soluble lignin (Dence, 1992). The concentrations of monomeric sugars in the soluble fraction were determined by HPLC using a BIORAD HPX87H column at 45°C, eluted at the 0.6 ml/min with 0.005 mol/l sulfuric acid. Sugars were detected in a 30°C-temperature controlled RI detector (Knauer HPLC pump and detector). In these conditions, xylose, mannose and galactose eluted at the same retention time were integrated as a single peak. Glucose, xylose, arabinose and acetic acid were used as external calibration standards. No corrections were performed due to sugar degradation reactions during acid hydrolysis. The factors used to convert sugar monomers to anhydromonomers were 0.90 for glucose and 0.88 for xylose and arabinose. Acetyl content was calculated as the acetic acid content multiplied by 0.7. These factors were calculated based on water addition to polysaccharides during acid hydrolysis (Irick et al.,

Table 1
Chemical composition and weight losses of *P. radiata* biodegraded by white- and brown-rot fungi

Sample number	Fungal species	Biodegradation time (days)	Weight loss (%) (std)	Wood component (% of extractive free, dry wood)							
				Glucan	Polyoses	Arabinan	Acetyl	Insoluble lignin	Soluble lignin	Total lignin	Sum
1	None (control)	–	0.0	41.9	19.0	1.1	1.3	27.5	0.6	28.1	91.4
2	*Trametes*	45	10.8 (0.8)	44.9	18.9	0.9	1.5	25.7	0.9	26.6	92.8
3	*versicolor*	77	22 (1)	47.4	19.0	0.8	0.7	24.1	1.2	25.3	93.2
4	*Punctularia*	30	0.4 (0.1)	43.7	19.4	1.2	1.5	27.8	0.6	28.4	94.2
5	*artropurpurascens*	45	0.7 (0.2)	44.4	19.1	1.1	1.4	26.0	0.7	26.7	92.7
6		90	5.7 (0.7)	46.7	19.1	1.0	1.1	26.1	0.7	26.8	94.7
7	*Poria*	30	1.0 (1.0)	43.9	18.8	0.9	1.2	27.4	0.7	28.1	92.9
8	*medula-panis*	45	4.6 (0.3)	43.5	18.2	1.0	1.1	26.7	0.6	27.3	91.1
9		90	15 (1)	44.8	18.9	0.8	0.8	26.2	0.8	27.0	92.3
10	*Merulius*	45	14 (2)	45.9	18.3	0.9	1.3	25.2	0.9	26.1	92.5
11	*tremellosus*	90	17.2 (0.9)	47.6	18.7	0.7	1.5	23.6	0.9	24.5	93.0
12	*Cerisporiopsis*	90	17.2 (0.8)	51.4	17.9	0.8	1.3	22.3	1.0	23.3	94.7
13	*subvermispora*	200	36 (2)	48.8	18.0	0.6	0.8	22.5	1.3	23.8	92.0
14	*Ganoderma*	140	20 (1)	44.7	17.2	1.1	1.4	24.8	0.4	25.2	89.6
15	*aplanatum*	360	31 (4)	45.4	19.0	0.6	1.2	25.7	0.8	26.5	92.7
16	*Poria*	30	6.1 (0.9)	46.1	16.7	0.8	1.3	28.1	0.7	28.8	93.7
17	*cocos*	45	8.1 (0.6)	44.1	16.3	0.7	1.2	30.3	0.6	30.9	93.2
18		90	9.4 (0.4)	43.8	16.2	0.7	1.0	29.3	0.8	30.1	91.8
19	*Laetiporus*	30	1.2 (0.1)	43.5	19.1	0.9	1.1	28.4	0.6	29.0	93.6
20	*sulfureus*	45	2.4 (0.6)	44.0	18.2	1.1	2.1	26.8	0.6	27.4	92.8
21		90	7.3 (0.7)	42.8	17.5	0.7	0.8	28.0	0.6	28.6	90.4
Minimal value		–	0.4	41.9	16.2	0.6	0.8	22.3	0.4	23.3	89.6
Maximal value		–	36	51.4	19.4	1.2	2.1	30.3	1.3	30.9	94.7
Average value		–	11.5	45.2	18.3	0.9	1.2	26.3	0.8	27.1	92.6
Minimal analytical deviation		–	–	0.0	0	0	0	0.02	0.01	0.03	–
Maximal analytical deviation		–	–	1.5	0.6	0.3	1.1	0.4	0.13	0.5	–
Average analytical deviation		–	–	0.4	0.2	0.1	0.2	0.2	0.04	0.2	–

1988; Kaar and Brink, 1991; Kaar et al., 1991; Laver and Wilson, 1993).

2.5. Analysis of wood samples by DRIFT spectroscopy

The 100 μm-sieved sample was dried at 100°C for 2 h and stored under P_2O_5. Twenty five mg of sample was homogenized with 225 mg of KBr for 1 min and put in a macro-cup of the Spike Technologies attachment for DRIFT spectroscopy. FTIR spectra were recorded between 4000 and 800 cm^{-1} in a Perkin Elmer FT-2000 FTIR spectrometer using 64 scans, triangular apodization and resolution of 4 cm^{-1}. Data points collection was set at the same resolution value (each 4 cm^{-1}) giving 795 data points for each FTIR recorded between 4000 and 824 cm^{-1}. Reflectance spectra were transformed to Kubelka-Munk (KM) units to minimize scattering contributions to the absorption measured (Michell, 1988; Anderson et al., 1991; Martens and Naes, 1989). The baseline was corrected to the regions near 800, 2000 and 3800 cm^{-1} and the spectrum normalized to the band nearest to 1510 cm^{-1}.

2.6. Principal component regression and partial least squares modeling

Spectral and chemical composition data were analyzed with the software package QUANT + available with the Perkin Elmer spectrometer. Spectral data were analyzed in digitized form at regular intervals of 4 cm^{-1}. Normalized spectra were mean scaled (covariance about the mean) by subtracting each sample spectrum from the mean spectrum (Martens and Naes, 1989; QUANT + guide book, Perkin Elmer, 1992). For partial least squares (PLS) modeling, property values (wood component concentrations) were also mean scaled. PLS models were based on the PLS-2 algorithm available in the QUANT +. This algorithm calculates regression

Table 2
Chemical composition and weight losses of *E. globulus* bidegraded by white- and brown-rot fungi

Sample number	Fungal species	Biodegradation time (days)	Weight loss (%) (std)	Wood component (% of extractive free, dry weight)						
				Glucan	Polyoses	Acetyl	Insoluble lignin	Soluble lignin	Total lignin	Sum
1	None (control)	–	0.0	45.3	15.7	2.7	22.4	4.5	26.9	90.6
2	*Trametes*	45	15.5 (0.4)	44.1	15.8	2.6	23.3	4.5	27.8	90.3
3	*versicolor*	90	31 (3)	41.6	14.3	2.7	23.3	3.7	27	85.6
4	*Punctularia*	45	1.7 (0.2)	44.4	15.7	2.5	22.2	4.7	26.9	89.5
5	*artropurpurascens*	90	6.2 (0.1)	44.4	14.2	2.7	21.0	4.2	25.2	86.5
6	*Poria*	45	4.6 (0.6)	42.6	14.8	2.7	22.4	3.7	26.1	86.2
7	*medula-panis*	90	7.5 (0.4)	42.8	13.9	2.3	22.6	4.3	26.9	85.9
8	*Merulius*	45	11 (3)	47.2	14.6	2.3	19.4	4.3	23.7	87.8
9	*tremellosus*	90	13.7 (0.9)	42.6	14.3	2.6	23.4	3.7	27.1	86.6
10	*Cerisporiopsis*	90	12 (2)	46.1	14.3	2.4	16.7	3.9	20.6	83.4
11	*subvermispora*	150	42 (3)	47.3	16.3	2.7	18.8	4.3	23.1	89.4
12	*Poria*	45	2.4 (0.2)	45.6	15.2	2.4	22.4	4.5	26.9	90.1
13	*cocos*	90	6.7 (0.5)	42.8	13.1	3.1	23.9	3.7	27.6	86.6
Minimal value		–	1.7	41.6	13.1	2.3	16.7	3.7	20.6	83.4
Maximal value		–	42	47.3	16.3	3.1	26.9	4.5	27.8	90.6
Average value		–	12.8	44.4	14.8	2.6	21.7	4.2	25.8	87.6
Minimal analytical deviation			–	0	0	0	0.06	0.01	0.1	–
Maximal analytical deviation			–	2.6	0.9	0.7	0.8	0.2	0.9	–
Average analytical deviation			–	0.5	0.3	0.3	0.2	0.1	0.2	–

models for all properties (wood component concentrations) simultaneously and is indicated for samples where there is a high degree of correlation among the properties (Martens and Naes, 1989; QUANT + guide book, Perkin Elmer, 1992). Principal component regression (PCR) models were based on principal component analysis (PCA) performed on spectral information followed by multiple linear regression (MLR) between each wood component concentration and chosen principal components. The analysis of variance (ANOVA) for each model gave R^2 and F values that were used to estimate the quality and validity of the models, respectively (Box and Wetz, 1973). Cross-validation was used to check standard outliers in both PLS and PCR models, but the reported data correspond to all samples in the calibration data set. The mathematical basis for multivariate calibration using PCR and PLS algorithms are detailed in tutorial papers (Beebe and Kowalski, 1987; Grahan, 1993; Olsson et al., 1995).

3. Results and discussion

3.1. Chemical and spectroscopic characterization of decayed wood samples

P. radiata softwood and *E. globulus* hardwood chips were degraded by a variety of white- and brown-rot fungi for periods of 30 days–1 year. Chemical composition of decayed wood samples was determined by wet chemical analysis. Among 21 samples of *P. radiata*, the glucan, polyoses (xylan + mannan + galactan) and total lignin concentrations ranged between 41.9–51.4%, 16.2–19.4%, and 23.3–30.9%, respectively (Table 1). Arabinan and acetyl contents were low in *P. radiata* wood and ranged between 0.6–1.2%, and 0.8–2.1%, respectively. Average analytical deviations owing to the wet chemical determination of wood components were approximately 1.0% of the component concentration value for glucan, 3% for polyoses and 2% for total lignin. In the case of arabinan and acetyl determinations, average analytical deviations were high and corresponded to 11% and 16%, respectively. High analytical deviations for the arabinan and acetyl contents are not surprising since low concentrations of arabinose and acetic acid are poorly detected by a HPLC-RI detector. Summative analysis indicated an average value of 92.6% for detected components in *P. radiata*. The remaining 7.4% should be accounted for as unanalyzed components such as uronic acids and ash, and also to sugar losses owing to side degradation reactions occurred during hot acid hydrolysis of wood samples (Kaar et al., 1991).

Glucan concentrations in *E. globulus* ranged from 41.6% to 47.3%. Polyoses and total lignin ranged from 13.1% to 16.3% and from 20.6% to 27.8%, respectively (Table 2). As expected, acetyl contents were higher in the hardwood than in the softwood and ranged between 2.3% and 3.1% in the former. Analytical deviations were

low for major wood components (glucan, polyoses and total lignin) and around 10% for acetyl determinations as also observed for *P. radiata* data. Summative analysis showed an average value of 87.6% for *E. globulus*. 4-*O*-methylglucuronic acid, which was not determined in this work, occurs at high concentrations in *E. globulus*, reaching values of 4.6% (Wallis et al., 1996). Sugar losses during acid hydrolysis, ash content and arabinan (detected in the range of 0–0.5% in these samples) should account for the remaining 7.8% of undetected compounds.

Data sets presented in Tables 1 and 2 can be considered as representative of chemical compositions expected for decayed wood samples when white- and brown-rot fungi are involved in the biodegradation process. Weight losses were in the range of 0.4–36% for the softwood and 1.7–42% for the hardwood. These weight losses covered low, medium and high extension of wood biodegradation. Also, different patterns of lignin and polysaccharides degradation were obtained. For example, selective lignin degradation was observed in samples decayed by *C. subvermispora* where the lowest total lignin concentrations were detected (23.3% in *P. radiata* and 20.6% in *E. globulus* samples decayed for 90 days). Preferential polysaccharide degradation was observed in samples decayed by the brown-rot fungus *P. cocos* where glucan and polyoses concentrations decreased with biodegradation time (Tables 1 and 2).

All decayed wood samples were also analyzed by DRIFT spectroscopy. Based on a previous optimization of spectra reproducibility (data not shown), 100 μm-sieved samples were dried and mixed with KBr to a final concentration of 10%. This procedure provided satisfactory reproducibility among the spectra as illustrated in Fig. 1 for two replicate samples of undecayed *P. radiata*. However, the bands at 3300, 2940 and 1100 cm^{-1} suffered from some lack of reproducibility. Although appropriate for opaque materials such as wood, the DRIFT technique can introduce anomalies into the IR spectrum of wood. In some cases, the bands near 1100 cm^{-1} can disappear completely (Anderson et al., 1991; Michell, 1994). These anomalies have been assigned to the scattering effect caused by the irregular surface of wood. The use of finely divided samples (granule size lower than 100 μm) and dilution in KBr can minimize the scattering effect. For instance, the spectra reported in this paper were similar to the transmission spectrum of thin wood sections (Michell, 1988; Ferraz et al., 1998a).

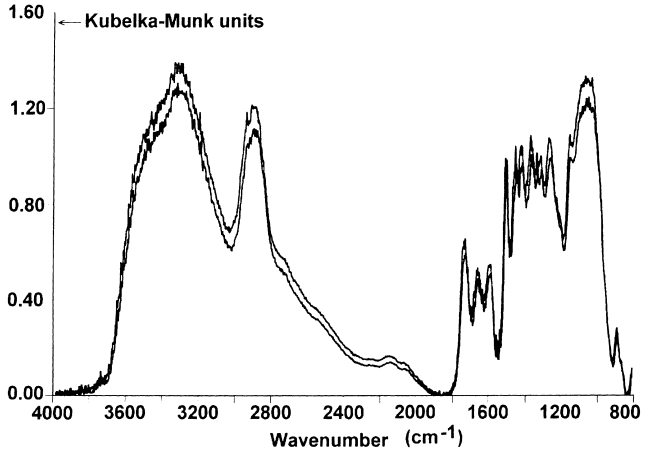

Fig. 1. DRIFT spectra of undecayed *P. radiata*. Samples were prepared separately and the spectra show the reproducibility between the two replicates.

FTIR spectra of wood samples contain several overlapped bands. Most of the wood components contribute, at least in part, to all observed bands (Anderson et al., 1991; Michell, 1988; Owen and Thomas, 1989). The only "pure" band is related to the aromatic moieties present in lignin, which gives a characteristic absorption near 1510 cm^{-1} and usually is used as a reference band (Michell, 1988). The assignment of bands in FTIR spectra of wood and its isolated components has been discussed (Anderson et al., 1991; Faix, 1992; Hortling et al., 1992; Michell, 1988; Owen and Thomas, 1989). The bands observed in *P. radiata* and *E. globulus* spectra agreed with reported bands for soft and hardwoods, respectively.

DRIFT spectra of representative white- and brown-rotted *P. radiata* wood samples were compared (Fig. 2). Fig. 2(a) shows the spectra of undecayed *P. radiata* and the sample decayed for 90 days by *C. subvermispora*. This decayed sample presented the lowest lignin concentration among the 21 samples studied (Table 1). As the spectra were normalized to the band at 1510 cm^{-1}, the increase in intensity of the bands at 1200–1000 cm^{-1} (C–O bands) could be related to a relative increase in the glucan content from 41.9% in the undecayed control to 51.4% in the decayed sample (Table 1). Considering the diminished polyoses content of decayed samples, the increase in the intensity of the band at 1750 cm^{-1} (unconjugated C═O band) could be related to new unconjugated acids present in the side chain of the lignin macromolecule or to the biodegradation-resistance of uronic acids- and acetyl-branched structures in polyoses. Increases in the 1660 cm^{-1} band (conjugated C═O) intensity in the *C. subvermispora*-decayed sample could be related to the occurrence of new conjugated carbonyl structures in the residual lignin of decayed wood. Other *P. radiata* samples where selective lignin biodegradation was achieved (e.g., sample decayed by *P. artropurpurascens* for 45 days) also presented the same pattern of changes in the FTIR spectra. However, the changes in the band intensities were less pronounced.

The spectra of undecayed *P. radiata* and a sample decayed by *P. cocos* for 45 days are shown in Fig. 2(b). This sample, which represents a typical brown-rot decay, had the highest lignin and one of the lowest poly-

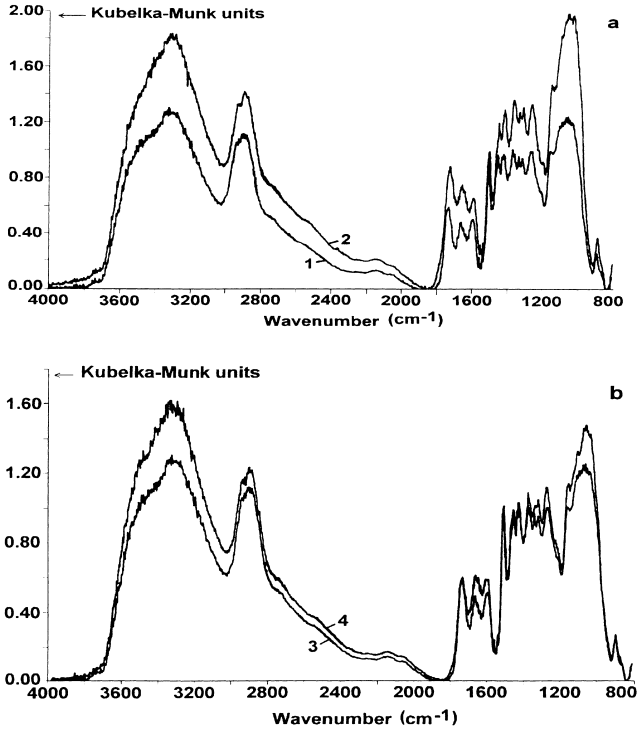

Fig. 2. (a) DRIFT spectra of undecayed *P. radiata* (1) and a sample decayed for 90 days by *Cerisporiopsis subvermispora* (2); (b) DRIFT spectra of undecayed *P. radiata* (3) and a sample decayed for 45 days by *Poria cocos* (4).

oses concentrations among the 21 samples studied (Table 1). In this case, the small increase in the 1200–1000 cm^{-1} band intensity could be related to a higher glucan content as compared to the wood control (41.9% in the undecayed *P. radiata* and 44.1% in the decayed sample). The band at 1750 cm^{-1} presented almost the same intensity in both the decayed sample and the control, while the polyoses content diminished in the former (Table 1). This fact plus the intensity increase in the 1660 cm^{-1} band suggested that brown-rot decay promotes the formation of new conjugated and unconjugated acid substructures in the side chain of the lignin macromolecule.

3.2. Multivariate calibration between spectral information and wood component concentrations

Monitoring structural or compositional wood changes by analysis of isolated bands in the FTIR spectrum is difficult, hence, the use of multivariate analysis provides the possibility of analyzing the entire spectral information simultaneously (Faix and Bottcher, 1993; Michell, 1994; Olsson et al., 1995). Duplicate spectra from the 21 samples of *P. radiata* listed in Table 1 were used as the data set for a multivariate calibration between spectral information and the contents of the major chemical components determined by wet chemical analysis. The entire spectral data (4000–824 cm^{-1}) or data from 1900 to 824 cm^{-1} were regressed against the contents of wood components using PCR or PLS algorithms. The analysis of variance of the calculated models (r^2 and F-statistic values) and critical F-values for 99% confidence level are presented in Table 3. The number of factors used in each model were chosen based on the cutoff point defined as the number of factors closest to the 10% significance level plus one. In both PCR and PLS models the number of factors used in each model were not lower than 6 or higher than 8 (Table 3). Direct comparison among models is restricted since different degrees of freedom, owing to the use of different number of factors in each model, are involved. However, critical F-values are similar for the degrees of freedom involved in these calibrations and a comparison among F-statistic and r^2 values indicated that the best models were obtained by PLS modeling. Also, more robust calibrations were obtained using the complete spectral data set instead of the low wavenumber region, suggesting a significant contribution of the O–H and C–H bands to the final model. These facts can be explained based on the calibration methods used. PCR model arises from a two-step procedure. The first one involves a PCA on the spectral data matrix. The second step provides a MLR between sample scores for the chosen principal components against the property studied (concentration of one wood component). On the other hand, PLS models use

Table 3
Analysis of variance of PCR and PLS models from multivariate calibration on diffuse reflectance FTIR spectral data and wood composition of *P. radiata* biodegraded by white- and brown-rot fungi[a]

	Wood component								
	Total lignin			Glucan			Polyoses		
	r^2	F-stat	Factors in the model	r^2	F-stat	Factors in the model	r^2	F-stat	Factors in the model
PCR models									
1900–824 cm^{-1}	0.88	41.9	6	0.68	12.49	6	0.75	17.8	6
4000–824 cm^{-1}	0.91	57.2	6	0.69	12.9	6	0.44	4.6	6
PLS models									
1900–824 cm^{-1}	0.96	103.3	7	0.94	72.1	7	0.80	19.8	7
4000–824 cm^{-1}	0.96	107.5	8	0.97	116.2	8	0.86	31.0	7

[a] Critical F-values: $F_{0.99;5,36} = 3.51$; $F_{0.99;6,35} = 3.29$; $F_{0.99;7,34} = 3.12$.

spectral data that are weighed by the concentrations of wood components in the calibration samples being studied. For this reason PLS variables are more highly correlated with wood component concentrations than principal components (Beebe and Kowalski, 1987; Grahan, 1993). Hence, only spectral information that relates to property variation will be accounted for. Therefore, the PLS calibration can avoid noise and scattering contributions to the final model.

The PLS models for entire FTIR spectral information provided a good estimate for all major wood component concentrations in *P. radiata* (Fig. 3). The average, maximum and minimum residue values for each predicted wood component concentration are shown in Table 4. The average errors in prediction of wood component contents were low and confirmed the goodness-of-fit of the PLS models. Only for comparison purposes one can observe that the prediction errors were slightly higher than the experimental deviations observed for the analytical data from the wet chemical analysis (Table 1). However, for polyoses determination, wet chemical analysis rarely provides analytical deviations higher than 0.6%, while errors of up to 1.2% were observed for the values predicted by PLS models (Tables 1 and 4).

Table 4
Average, minimum and maximal errors of prediction of wood component concentration in *P. radiata* biodegraded by white- and brown-rot fungi, using PLS models based on diffuse reflectance FTIR spectral data

Wood component	Average error of prediction (%)	Minimum error of prediction (%)	Maximum error of prediction (%)
Total lignin	0.3	0.01	0.8
Glucan	0.3	0.01	1.2
Polyoses	0.3	0.00	1.2

In order to evaluate the regions of the spectra that were significant for the PLS models, the loadings of the first two factors (latent variables) were plotted as a function of the wavenumber (Fig. 4). The first and second factors explained 89.0% and 6.3% of the spectral

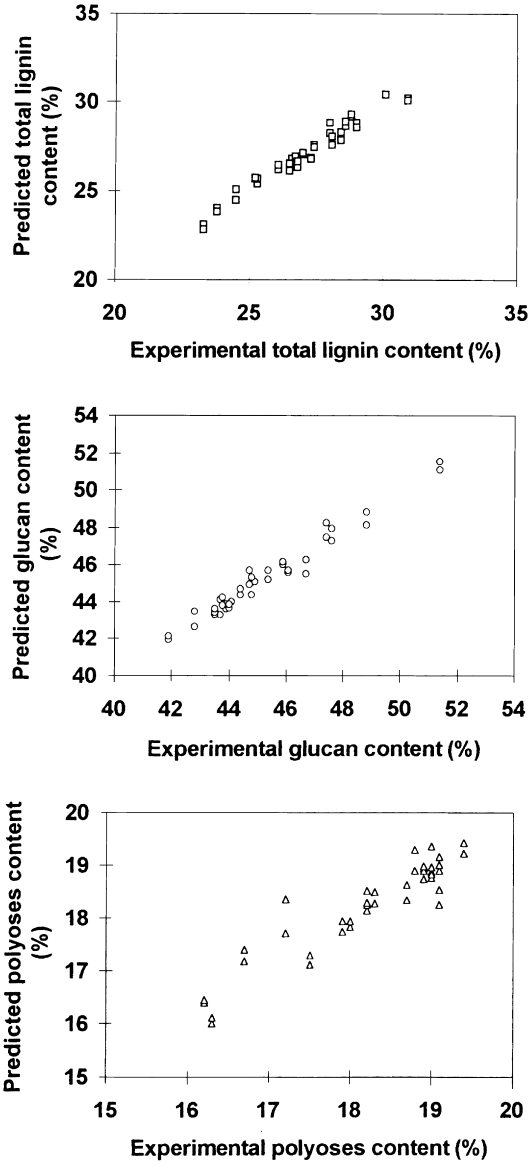

Fig. 3. Predicted (PLS models) wood component concentrations in *P. radiata* vs experimentally determined values (wet chemistry analysis).

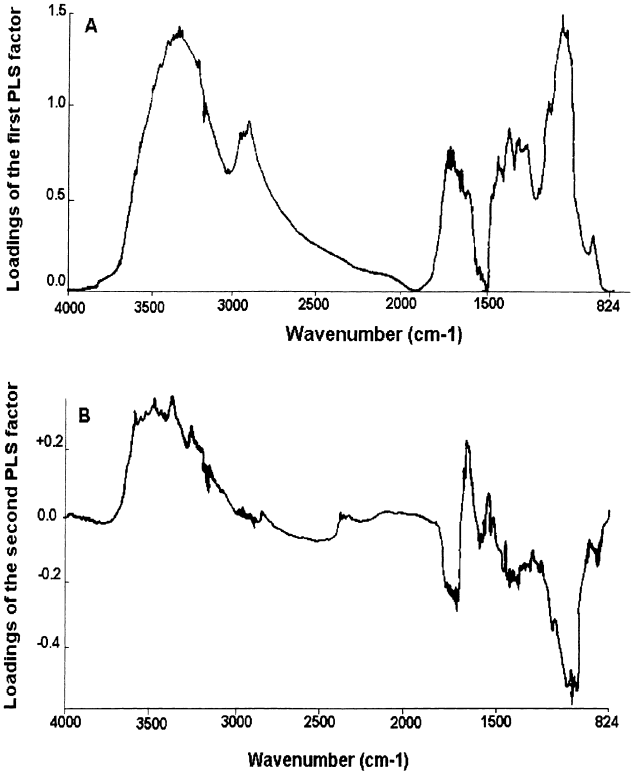

Fig. 4. Loadings of the first (a) and the second (b) principal components of PLS models relating FTIR spectral data with chemical composition in decayed *P. radiata* wood samples.

variance, respectively. The first factor (Fig. 4(a)) presented loading values similar to that of an average FTIR spectrum, except for the 1510 cm^{-1} band which was normalized in all the spectra. The second factor (Fig. 4(b)) presented loadings with one third of the first factor magnitude. The second factor also presented positive and negative loadings where bands near 3300, 2940, 1665, 1660 cm^{-1} were positive and bands near 1750, 1470 and 1100 cm^{-1} were negative. The plots shown in Fig. 4 indicate that there is no specific region in the spectra contributing to the PLS model. Instead, the loading values were almost proportional to the signal intensity observed in the spectra recorded in KM units.

PLS models for entire spectral data were also calculated to predict wood component concentrations in *E. globulus* decayed samples. F-statistics and r^2 values for total lignin, glucan and polyoses models were 37.6 and 0.93, 31.2 and 0.91, and 7.1 and 0.70, respectively. These models (using 6 factors) were significant at the 99% confidence level (critical $F_{0.99;\,6,26} = 3.85$). However, the PLS model for xylan prediction presented a poor r^2 and a F-statistic only slightly higher than the critical F-value. The predicted wood component concentrations in *E. globulus* (PLS model) vs experimentally determined values (wet chemistry analysis) are shown in Fig. 5. In all cases the r^2 and F-statistics values were lower than those observed for *P. radiata* models. There is no direct explanation for the lower quality of the models obtained for *E. globulus* wood. One reason could be the smaller wood component concentration ranges as compared with samples of *P. radiata* (Tables 2 and 1, respectively). Besides the structural differences in lignin and polyoses, a significant difference in the chemical composition of the *P. radiata* and *E. globulus* samples was the amount of extractives. In the *P. radiata* samples the amount of extractives was never higher than 1%. In the *E. globulus* samples, the values ranged from 1.5% to 4.6% and the average was 3.1%. Wood extractives contain a complex mixture of waxes and phenolic compounds. All these compounds can absorb in the IR region and overlap the signals of the major wood components. Probably, these overlapped signals can affect the PLS models, since some spectral information due to the extractives could be accounted for in the major wood component models. Therefore, for samples with high amounts of extractives, better models could be obtained by using previously extracted samples for recording the FTIR spectrum.

3.3. Validation of the FTIR models

Model validation was performed on the *P. radiata* data. In a first approach, three samples were left out randomly and a series of new models were calculated (Table 5). Samples left out were then used as test groups (Table 6). Models with 3 samples out continued to give F values significant at the 0.01 level and high R^2 values.

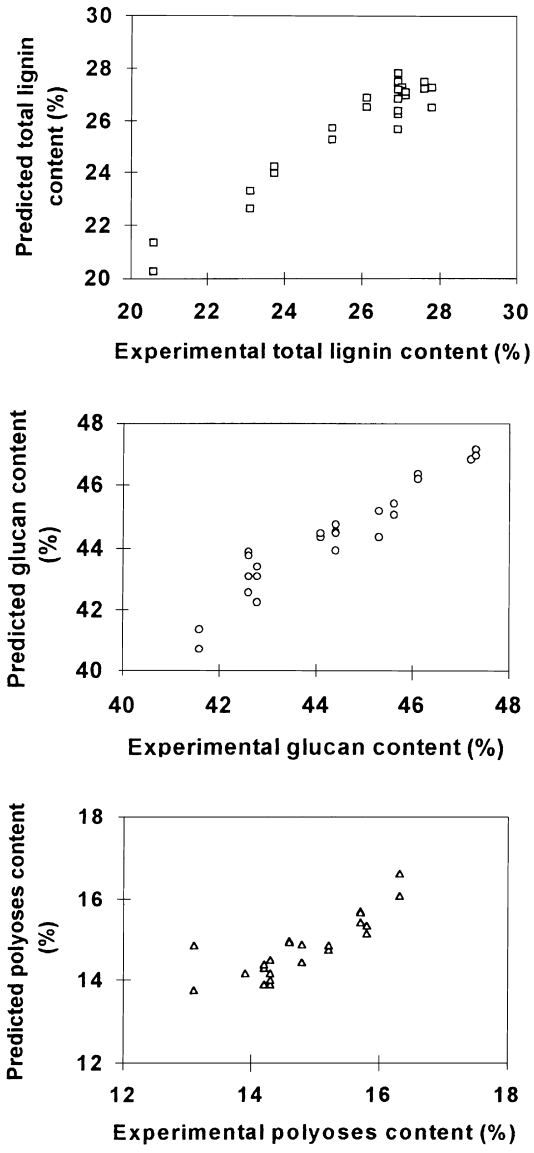

Fig. 5. Predicted (PLS models) wood component concentrations in *E. globulus* vs experimentally determined values (wet chemistry analysis).

In general, predicted values for samples left out were in good agreement with experimental data. However, for a limited number of models the predicted wood component concentrations for some specific samples presented high deviations. For example, model 4 was not adequate to predict glucan and total lignin concentrations in sample 13 (Table 6). A similar problem was observed in model 7 on prediction of wood component concentrations in sample 12. These samples presented the lowest lignin and the highest glucan concentrations in the data set. Leaving out these samples, the resulting model was not suitable to predict wood component concentrations at that concentration range. This result was also observed using the one-out cross validation technique (data not shown). As mentioned before, different fungal species and varied biodegradation times were used to

Table 5
Validation models for PLS calibration on DRIFT spectral data and wood composition of *P. radiata* biodegraded by white- and brown-rot fungi

Model number	Samples out	Glucan			Polyoses			Total lignin		
		R^2	F	Factors	R^2	F	Factors	R^2	F	Factors
T	None	0.97	116.2	8	0.86	31.0	7	0.96	107.5	8
1	1, 15, 16	0.98	202.0	8	0.88	28.5	7	0.96	117.9	6
2	4, 6, 21	0.98	168.6	8	0.84	21.7	7	0.97	109.5	8
3	17, 19, 20	0.99	700.2	12	0.98	139.8	11	0.99	204.0	10
4	5, 8, 13	0.96	91.2	8	0.89	31.4	7	0.96	113.9	6
5	6, 16, 19	0.92	58.5	6	0.75	14.6	6	0.89	51.1	5
6	2, 18, 20	0.88	30.0	7	0.79	14.9	7	0.96	89.0	7
7	10, 12, 16	0.96	83.5	8	0.89	32.7	7	0.97	115.3	8
8	1, 8, 18	0.97	95.1	8	0.83	19.7	7	0.96	88.3	7
9	11, 14, 17	0.97	128.7	8	0.88	28.3	7	0.97	131.4	8
10	3, 7, 9	0.93	52.8	7	0.70	11.1	6	0.97	127.6	7

Table 6
PLS-predicted wood component concentrations in *P. radiata* biodegraded by white- and brown-rot fungi using validation models performed on FTIR spectral data

Model number	Sample left out	Component concentration (% of extractive free, dry weight)								
		Glucan			Polyoses			Total lignin		
		Exp[a]	Predicted[b]	*D*	Exp	Predicted	*D*	Exp	Predicted	*D*
1	1a	41.9	43.2	−1.3	19	19.5	−0.5	28.1	28.3	−0.2
	1b	41.9	42.4	−0.5	19	19	0	28.1	29.1	−1
	16a	46.1	43.7	2.4	16.7	17.8	−1.1	28.8	29.5	−0.7
	16b	46.1	43.4	2.7	16.7	18	−1.3	28.8	29.7	−0.9
	15a	45.4	47.1	−1.7	19	19.2	−0.2	26.5	25.9	0.6
	15b	45.4	48.1	−2.7	19	19.7	−0.7	26.5	25.4	1.1
2	4a	43.7	43.1	0.6	19.4	19.2	0.2	28.4	27.5	0.9
	4b	43.7	44	−0.3	19.4	18.9	0.5	28.4	28	0.4
	6a	46.7	44.7	2	19.1	18.9	0.2	26.8	26.2	0.6
	6b	46.7	44.3	2.4	19.1	19	0.1	26.8	26.7	0.1
	21a	42.8	44.3	−1.5	17.5	17.2	0.3	28.6	28.6	0
	21b	42.8	44.3	−1.5	17.5	17.3	0.2	28.6	28.8	−0.2
3	17a	44.1	43.6	0.5	16.3	16.5	−0.2	30.9	29.2	1.7
	17b	44.1	43.7	0.4	16.3	16.6	−0.3	30.9	29.1	1.8
	19a	43.5	43.1	0.4	19.1	17.5	1.6	29	28.8	0.2
	19b	43.5	43.7	−0.2	19.1	17.5	1.6	29	28.9	0.1
	20a	44	43.2	0.8	18.2	17.4	0.8	27.4	27.6	−0.2
	20b	44	43.3	0.7	18.2	17.5	0.7	27.4	27.6	−0.2
4	5a	44.4	44	0.4	19.1	17.9	1.2	26.7	27.5	−0.8
	5b	44.4	44.9	−0.5	19.1	17.8	1.3	26.7	27.3	−0.6
	8a	43.5	45.5	−2	18.2	17.8	0.4	27.3	26.3	1
	8b	43.5	44.3	−0.8	18.2	18.1	0.1	27.3	26.6	0.7
	13a	48.8	51.8	−3	18	18.5	−0.5	23.8	21.1	2.7
	13b	48.8	53.9	−5.1	18	18.2	−0.2	23.8	21.1	2.7
5	6a	46.7	45	1.7	19.1	18.6	0.5	26.8	26.4	0.4
	6b	46.7	44.8	1.9	19.1	18.6	0.5	26.8	26.8	0
	16a	46.1	43.4	2.7	16.7	17.6	−0.9	28.8	29.8	−1
	16b	46.1	42.9	3.2	16.7	17.8	−1.1	28.8	29.6	−0.8
	19a	43.5	42.5	1	19.1	17.9	1.2	29	29.5	−0.5
	19b	43.5	42.9	0.6	19.1	18.1	1	29	29.3	−0.3
6	2a	44.9	45.7	−0.8	18.9	18.7	0.2	26.6	26.7	−0.1
	2b	44.9	44.5	0.4	18.9	18.7	0.2	26.6	27	−0.4
	18a	43.8	44.8	−1	16.2	16.9	−0.7	30.1	30.2	−0.1
	18b	43.8	44.8	−1	16.2	17.1	−0.9	30.1	29.7	0.4
	20a	44	43.8	0.2	18.2	18.2	0	27.4	28.2	−0.8
	20b	44	43.8	0.2	18.2	18.3	−0.1	27.4	28.1	−0.7

Table 6 (Continued)

Model number	Sample left out	Component concentration (% of extractive free, dry weight)								
		Glucan			Polyoses			Total lignin		
		Exp[a]	Predicted[b]	*D*	Exp	Predicted	*D*	Exp	Predicted	*D*
7	10a	45.9	46.8	−0.9	18.3	18.4	−0.1	26.1	26.1	0
	10b	45.9	46.2	−0.3	18.3	18.5	−0.2	26.1	26.5	−0.4
	12a	51.4	47.7	3.7	17.9	17.7	0.2	23.3	24	−0.7
	12b	51.4	47.8	3.6	17.9	17.9	0	23.3	23.6	−0.3
	16a	46.1	43.1	3	16.7	17.7	−1	28.8	29.6	−0.8
	16b	46.1	43.8	2.3	16.7	18	−1.3	28.8	29.7	−0.9
8	1a	41.9	42.2	−0.3	19	18.7	0.3	28	28.9	−0.9
	1b	41.9	43	−1.1	19	19.2	−0.2	28	28.2	−0.2
	8a	43.5	44.7	−1.2	18.2	18.1	0.1	27.3	26.4	0.9
	8b	43.5	44.4	−0.9	18.2	18.5	−0.3	27.3	26.2	1.1
	18a	43.8	45	−1.2	16.2	16.9	−0.7	30.1	30.3	−0.2
	18b	43.8	44.7	−0.9	16.2	17	−0.8	30.1	30	0.1
9	11a	47.6	47	0.6	18.7	18.3	0.4	24.5	25.5	−1
	11b	47.6	47.9	−0.3	18.7	18.4	0.3	24.5	25	−0.5
	17a	44.1	43.8	0.3	16.3	16.6	−0.3	30.9	29.2	1.7
	17b	44.1	44.1	0	16.3	17.1	−0.8	30.9	29	1.9
	14a	44.7	45.3	−0.6	17.2	18.7	−1.5	25.2	25.6	−0.4
	14b	44.7	47.4	−2.7	17.2	18.7	−1.5	25.2	25.8	−0.6
10	3a	47.4	47.2	0.2	19	18.8	0.2	25.3	25.5	−0.2
	3b	47.4	46.7	0.7	19	18.7	0.3	25.3	25.9	−0.6
	7a	43.9	42.9	1	18.8	18.5	0.3	28.1	27.3	0.8
	7b	43.9	43	0.9	18.8	18.1	0.7	28.1	27.8	0.3
	9a	45.1	45.1	0	18.9	17.8	1.1	27	26.6	0.4
	9b	45.1	45.4	−0.3	18.9	17.8	1.1	27	26.7	0.3
Minimal deviation				0			0			0
Maximal deviation				5.1			1.6			2.7
Average deviation				1.3			0.6			0.7

[a] Exp = data from wet chemical analysis.
[b] Predicted = data predicted by the PLS model.

provide a data set covering all types of wood biodegradation patterns. However, selective lignin degradation is achieved only with some specialized fungal species such as *C. subvermispora*. Therefore, leaving out the few samples representing selective lignin degradation, results in a poor range of wood component concentrations prediction, despite that the models were useful in the mid range of wood component concentrations (Tables 5 and 6).

Another approach to validate the FTIR models was to evaluate component losses during a biotreatment. Component losses, that represents the mass balance for each individual wood component, are calculated based on the weight loss owing to the biotreatment and the concentration of each component before and after biodegradation (Blanchette et al., 1992). Weight and component losses as a function of time for two different fungal treatments are shown in Fig. 6. In this validation approach, some wood component concentrations were determined by conventional chemical analysis. In Fig. 6(a), the component losses calculated for samples decayed for 45 and 77 days are based on data from Table 1, samples 2 and 3. Similarly, in Fig. 6(b), the data for wood decay for 90 and 200 days are based on data from samples 12 and 13 (Table 1). The other component loss data were calculated based on the weight loss of each sample (Fig. 6) and the chemical compositions estimated based on FTIR spectral information and the previously developed PLS models. The overall curves for wood component losses are expected to show increasing loss values with time, since they represent a mass balance for each wood component. Based on Fig. 6 it is apparent that despite the method used for chemical composition estimation, the component losses values are progressive and fitted well to a typical unselective lignin degradation caused by *T. versicolor* (Fig. 6(a)) and to a selective lignin removal caused by *C. subvermispora* (Fig. 6(b)).

4. Conclusion

The multivariate calibration performed in this work permits estimation of wood component concentrations in decayed wood samples by FTIR spectroscopy. The application of this method depends on building a specific calibration set of decayed samples for each evaluated wood species. After this step, trials for evaluating wood biodegradation by a large number of fungal spe-

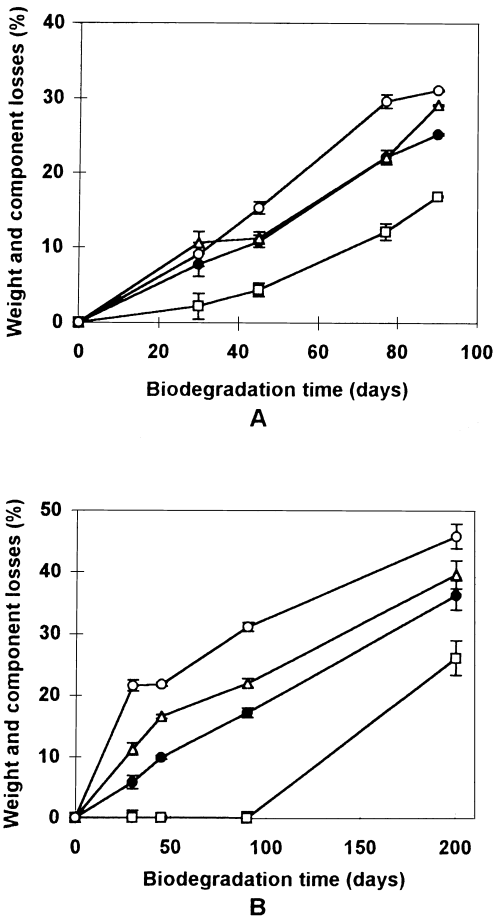

Fig. 6. Weight and component losses of *P. radiata* decayed by *Trametes versicolor* (A) and *Cerisporiopsis subvermispora* (B). Component losses for 45 and 77 days in Fig. 6(a) and 90 and 200 days in Fig. 6(b) were calculated from chemical compositions as reported in Table 1. The other component loss values were calculated based on the weight loss reported in each figure and the chemical compositions estimated based on FTIR spectral data. (–●–) weight, (–○–) lignin, (–Δ–) polyoses, and (–□–) glucan losses, respectively. Error bars are based on weight loss determinations from replicate cultures.

cies could be easily performed. FTIR estimation of wood composition in biodegraded samples could facilitate the selection of a reduced number of fungal species. With this procedure, only wood samples biodegraded by primarily selected fungal species should be further characterized by wet chemical analysis to confirm the FTIR-estimated results.

Acknowledgements

The authors thank M.E.M. Coelho for technical assistance. Helpful discussions and a critical reading of this manuscript by Dr. Roy E. Bruns and Dr. André Cotrim are also acknowledged. Financial support from FAPESP (grant number 95/04195-0 and contract number 94/0906-7), CNPq, FAENQUIL/SCTDE-SP, Fundación Andes-Chile (C-12345/9) and FONDECYT (1960227) is acknowledged.

References

Akhtar, M., Blanchette, R.A., Myers, G., Kirk, T.K., 1998. An Overview of Biomechanical Pulping Research. In: Young, R.A., Akhtar, M. (Eds.), Environmentally Friendly Technologies for the Pulp and Paper Industry. Wiley, New York, pp. 309–340.

Anderson, T.H., Weaver, F., William, F., Owen, N.L., 1991. Anomalies in diffuse reflectance infrared spectra of wood and wood polymers. J. Mol. Structure 249, 257–275.

Beebe, K.R., Kowalski, K., 1987. An introduction to multivariate calibration and analysis. Anal. Chem. 59, 1007A–1017A.

Bettucci, L., Speranza, M., Piaggio, M., 1992. Selecting white-rot fungi for biopulping. Proceedings of the Braz. Symp. Chem. Lignins and other Wood Components, vol 3, pp. 394–401.

Blanchette, R.A., Burnes, T.A., Eerdmans, M.M., Akhtar, M., 1992. Evaluating isolates of *Phanerochaete chrysosporium* and *Cerisporiopsis subvermispora* for use in biological pulping processes. Holzforschung 46, 109–115.

Blanchette, R.A., Otjen, L., Carlson, M.C., 1987. Lignin distribution in cell walls of birch wood decayed by white-rot basidiomycetes. Phytopathology 77, 684–690.

Box, G.E.P., Wetz, J., 1973. Criteria for judging adequacy of estimation by an approximate response function. University of Wisconsin Technical Report 9.

Chen, Y.R., Schimidt, E.L., 1995. Improving aspen kraft pulp by a novel low-technology fungal pretreatment. Wood Fiber Sci. 27, 198–204.

Dence, C.W., 1992. The determination of Lignin. In: Lin, Y.L., Dence, C.W. (Eds.), Methods in Lignin Chemistry. Springer, Berlin, pp. 33–62.

Enoki, A., Tanaka, H., Fuse, G., 1988. Degradation of lignin-related compounds, pure cellulose, and wood components by white-rot and brown-rot fungi. Holzforschung 42, 85–93.

Faix, O., 1992. Fourier Transform Infrared Spectroscopy. In: Lin, Y.L., Dence, C.W. (Eds.), Methods in Lignin Chemistry. Springer, Berlin, pp. 83–109.

Faix, O., Bottcher, J.H., 1993. Determination of phenolic hydroxyl group contents in milled wood lignins by FTIR spectroscopy applying partial-least squares (PLS) and principal component regression (PCR). Holzforschung 47, 45–49.

Ferraz, A., Christov, L., Akhtar, M., 1998a. Fungal pretreatment for organosolv pulping and dissolving pulping production. In: Young, R.A., Akhtar, M. (Eds.), Environmentally Friendly Technologies for the Pulp and Paper Industry. Wiley, New York, pp. 421–447.

Ferraz, A., Esposito, E., Bruns, R.E., Durán, N., 1998b. The use of principal component analysis (PCA) for pattern recognition in Eucalyptus grandis wood biodegradation experiments. W. J. Microbiol. Biotechnol. 14, 487–490.

Ferraz, A., Mendonça, R., Cotrim, A.R., Silva, F.T., 1996. The use of white-rot decay as a pretreatment for organosolv delignification *Eucalyptus grandis* wood. Proceedings of the Sixth International Conference of Biotechnol. Pulp and Paper Industry, pp. 221–224.

Ferraz, A., Baeza, J., Durán, N., 1991. Softwood biodegradation by an ascomycete *Chrysonilia sitophila* (TFB 27441 strain). Lett. Appl. Microbiol. 13, 86.

Grahan, R.C., 1993. Multiple regression and multivariate analysis: Data analysis for the Chemical Sciences – A guide to statistical techniques. VCR Publishers, New York.

Hortling, B., Forsskahal, I., Janson, J., Sundquist, J., Viikari, L., 1992. Investigations of fresh and biologically decayed birch. Holzforschung 46, 9–19.

Irick, T.J., West, K., Brownell, H.H., Schiwald, W., Saddler, J.N., 1988. Comparison of colorimetric and HPLC techniques for

quantitating the carbohydrate components of steam-treated wood. Appl. Biochem. Biotechnol. 17, 137–149.

Kaar, W.E., Brink, D.L., 1991. Summative analysis of nine common north american woods. J. Wood Chem. Technol. 11, 479–494.

Kaar, W.E., Gool, L.G., Merriman, M.M., Brink, D.L., 1991. The complete analysis of wood polysaccharides using HPLC. J. Wood Chem. Technol. 11, 447–463.

Laver, M.L., Wilson, K.P., 1993. Determination of carbohydrates in wood pulp products. Tappi J. 76 (6), 155–159.

Martens, H., Naes, T., 1989. Multivariate Calibration. Wiley, New York.

Messner, K., Srebotnik, E., 1994. Biopulping: An overview of developments in an environmentally safe papermaking technology. FEMS Microbiol. Rev. 13, 351–364.

Michell, A.J., 1988. Infra-red spectroscopy transformed – new applications in wood and pulping chemistry. Appita 41, 375–380.

Michell, A.J., 1994. Vibrational spectrometry – a rapid means of estimating plantation pulp wood quality. Appita 47, 29–37.

Olsson, R.J.O., Tomani, P., Karlsson, M., Joseffson, T., Sjoberg, K., Bjorklund, C., 1995. Multivariate characterization of chemical and physical descriptors in pulp using NIRR. Tappi J. 78 (10), 158–166.

Owen, N.L., Thomas, D.W., 1989. Infrared studies of hard and soft woods. Appl. Spectroscopy 43, 451–455.

Perez, V., Troya, M.T., Gonzalez-Villa, F.J., Arials, E., Gonzalez, A.E., 1993. In vitro decay of *Aextoxicon punctatum* and *Fagus sylvatica* woods by White- and Brown-rot Fungi. Wood Sci. Technol. 27, 295–307.

Reeves III, J.B., 1993. Infrared spectroscopic studies on forage and by-product fibre fractions and lignin determination residues. Vibrational Spectroscopy 5, 303–310.

Rodriguez, J., Ferraz, A., Nogueira, R.P., Ferrer, I., Esposito, E., Durán, N., 1997. Lignin biodegradation by the ascomycete *Chrysonilia sitophila*. Appl. Biochem. Biotechnol. 62, 233–242.

Setliff, E.C., Marton, R., Granzow, S.G., Eriksson, K.L., 1990. Biomechanical pulping with white-rot fungi. Tappi J. 73 (8), 141–147.

Schultz, T.P., Templeton, M.C., McGinnis, G.D., 1985. Rapid determination of lignocellulose by diffuse reflectance fourier transform infrared spectrometry. Anal. Chem. 57, 2867–2869.

Tseng, D.Y., Vir, R., Traina, S.J., Chalmers, J.J., 1996. A fourier transform infrared spectroscopic analysis of organic matter degradation in a bench-scale solid substrate fermentation (composting) system. Biotechnol. Bioeng. 52, 661–671.

Wallis, A.F.A., Wearne, R.H., Wright, P.J., 1996. Chemical analysis of polysaccharides in plantation eucalypt and pulps. Appita 49, 258–262.