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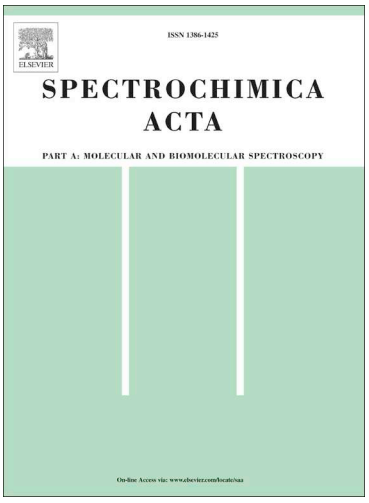

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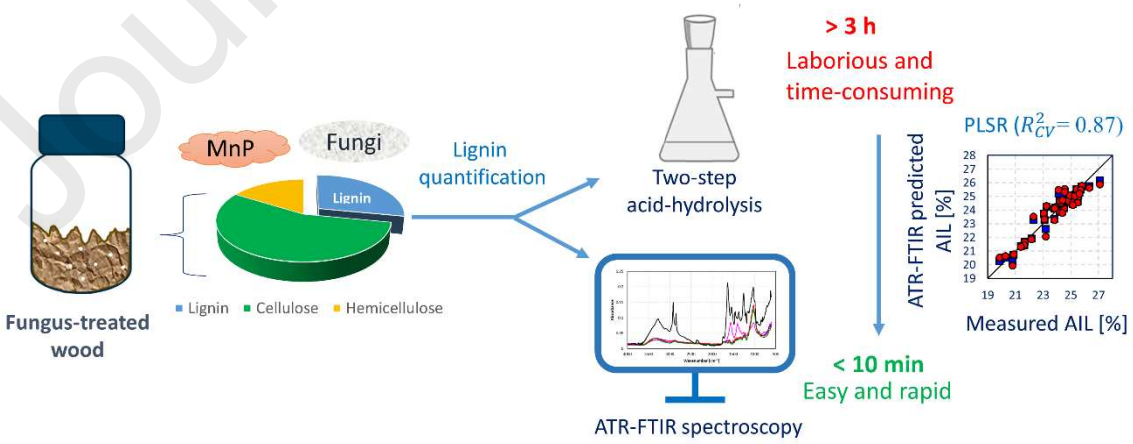
Abstract

Lignin determination in lignocellulose with the conventional two-step acid hydrolysis method is highly laborious and time-consuming. However, its quantification is crucial to monitor fungal pretreatment of wood, as the increase of acid-insoluble lignin (AIL) degradation linearly correlates with the achievable enzymatic saccharification yield. Therefore, in this study, a new attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy method was developed to track fungal delignification in an easy and rapid manner.

Partial least square regression (PLSR) with cross-validation (CV) was applied to correlate the ATR-FTIR spectra with the AIL content (19.9%–27.1%). After variable selection and normalization, a PLSR model with a high coefficient of determination (R^2_{CV} = 0.87) and a low root mean square ($RMSECV$ = 0.60%) were obtained despite the heterogeneous nature of the fungal solid-state fermentation. These results show that ATR-FTIR can reliably predict the AIL content in fungus-treated wood while being a high-throughput method. This novel method can facilitate the transition to the wood-based economy.

Graphical abstract



Keywords

Biobased economy; Lignin analysis; Manganese peroxidase; *Phanerochaete chrysosporium*; Solid-state fermentation; White-rot fungi

1 Introduction

The depletion of fossil resources resulted in increased interest in the search for an alternative source of energy and chemicals. Lignocellulosic biomass from plant cell walls is one of the most promising feedstocks for the production of biofuels and value-added biochemicals, due to its broad availability, renewable production and low cost [1]. Lignocellulose consists of three main compounds, i.e. the polysaccharides cellulose and hemicellulose and the polyaromatic compound lignin. In a sugar platform biorefinery, the degradation of this lignin has crucial importance in obtaining fermentable sugars from the polysaccharides [2]. Various pretreatment techniques, including physical, chemical, fungal, and physicochemical methods, have been employed to reduce the amount of lignin in lignocellulose. Fungal pretreatment, which mainly uses white-rot fungi for the degradation of lignin, is a pretreatment method that does not generate fermentation inhibiting by-products and is environmentally friendly with low chemical inputs [3]. *Phanerochaete chrysosporium* is one of the most widely studied white-rot fungi which produces manganese-peroxidase (MnP) and lignin peroxidase (LiP) for the degradation of lignin. In our previous study on the solid-state fermentation (SSF) of poplar wood by *P. chrysosporium*, a positive linear correlation was found between the acid-insoluble lignin (AIL) degradation and the obtained enzymatic saccharification yield which is in agreement with the findings of other studies [4,5]. Therefore, the monitoring of lignin has great importance in the optimization of the fungal pretreatment process.

The oldest and most popular method for lignin quantitation is the Klason method which has been outlined by the National Renewable Energy Laboratory (NREL) [6].

In this procedure, a two-step acid hydrolysis is performed on lignocellulose. First, the biomass is treated with concentrated sulphuric acid (72% w/w) at 30°C for 1 h, then the mixture is diluted with water to 4% w/w sulphuric acid and autoclaved at 121°C for 1 h to hydrolyse the polysaccharides into monomeric sugars. The acid-insoluble lignin (so-called Klason lignin) is gravimetrically quantified while the acid-soluble lignin is spectrophotometrically determined. Although this conventional wet chemistry method has been markedly useful for lignin quantification in lignocellulose, it is highly laborious, time-consuming (> 3 h) and requires the use of hazardous concentrated sulphuric acid and a relatively high amount of biomass (300 mg). Hence, the need is reinforced to develop an alternative method that allows rapid and easy lignin quantification with minimal sample preparation.

Fourier transform infrared (FTIR) analytical spectroscopy in the mid-infrared region (4000–400 cm^{-1}) has received increased interest in wood characterization due to the advances in the development of the attenuated total reflectance (ATR) technique [7,8]. In the traditional KBr pellet based FTIR analysis in transmission geometry, the additional preparation of pellets from infrared inactive KBr and the sample is required. In contrast, the ATR technique uses only minimal sample preparation, i.e., drying and homogenization (e.g. milling) after which the sample is directly pressed onto the ATR crystal and the spectral acquisition is performed within minutes allowing a very convenient analysis.

In the field of wood decay, ATR-FTIR has been recognized for its potential to assess the fungal deconstruction of lignocellulose [9,10]. Although it was demonstrated that

ATR-FTIR with multivariate data analysis such as partial least squares regression (PLSR) modelling can be applied for the quantitative determination of lignin in lignocellulose [11,12], its use for lignin estimation has not been evaluated yet in wood which was pretreated by white-rot fungi.

In this work, an ATR-FTIR method combined with PLSR was developed to determine the acid-insoluble lignin content in fungus-treated poplar wood hereby providing a simple and fast alternative (< 10 min) to the conventional labour- and time-intensive (> 3 h) two-step acid hydrolysis. This included the research subgoals of (1) obtaining a set of fungus-treated poplar wood samples with a wide range of AIL, (2) correlating the quantification of lignin in wood that is pretreated with white-rot fungi by ATR-FTIR coupled with PLSR to the traditional Klason lignin determination, (3) uncover and overcome possible interferences (4) optimize the PLSR model by variable selection and spectral pre-processing.

2 Materials and Methods

2.1 Lignocellulose substrate and white-rot fungi

Poplar wood sawdust was obtained from Sawmill Caluwaerts Willy (Holsbeek, BE). The particle size distribution of the sawdust was determined by sieve analysis. 86.1% w/w of the pellets were collected between the 2 mm and 0.075 mm screens. The white-rot fungus *Phanerochaete chrysosporium* MUCL 19343 was used for the solid-state fungal pretreatment studies. A spore suspension of $5 \cdot 10^6$ spores/mL (corresponds to 0.49 OD at 650 nm) was freshly prepared in distilled water from 5 days old cultures grown on potato dextrose agar at 39°C.

2.2 Fermentation media

Based on our previous study, the complex medium contained 3 g/L $NaNO_3$, 20 g/L glucose, 0.5 g/L KCl, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L $FeSO_4 \cdot 7H_2O$, 1 g/L KH_2PO_4, 0.34

g/L veratryl alcohol, 0.1% v/v Tween 80, 3.69 mM $CuSO_4$ and 1.41 mM $MnSO_4$ creating the optimal metal ion dosage of 2.01 µmol Cu^{2+}, 0.77 µmol Mn^{2+} g^{-1} dry weight (DW) wood for enhanced delignification [4,13]. The simplified media were composed of 3.69 mM $CuSO_4$, 1.41 mM $MnSO_4$ with or without 20 g/L glucose and/or 3 g/L $NaNO_3$.

2.3 Solid-state fungal pretreatment

Solid-state fermentation (SSF) of poplar wood was performed at different fermentation conditions to obtain pretreated poplar wood with varying lignin content for lignin calibration. These SSFs differed in the applied substrate sterilization, fermentation duration, medium composition and fermentation set-up (rolling bottles or trays).

SSF of sterilized wood in rolling bottles was performed in Schott bottles as described in our previous study [4]. Briefly, these fermentations were conducted in 100 mL Schott bottles closed with sterile-venting screw caps. The fermentation bottles contained 3.67 g dry weight poplar wood, 2 mL sterile media, 3.7 mL spore suspension ($5 \cdot 10^6$ spores/g DW wood) and distilled water creating a moisture content of 75% w/w on a wet basis. The media and the poplar wood were sterilized separately by autoclaving each at 121°C for 20 min. The SSF bottles were rolled at 4 rpm on a bottle roller (88881004 Bottle/Tube Roller, Thermo Scientific™) and incubated (TC 255 S, Tintometer Inc.) at 37°C for up to 4 weeks.

Tray fermentations of non-sterilized poplar wood were carried out in 500 mL crystallizing dishes containing 2 mL medium, 2.8 g DW untreated non-sterilized wood and 0.9 g DW pretreated wood as inoculum. For a detailed procedure see Section S1 in the supplementary materials. This inoculation technique was necessary to avoid the outcompetition of indigenous microbial communities with the white-rot fungi during

SSF of non-sterilized wood [14]. Tray fermentations were performed at 75% moisture content and 37°C for 4 weeks.

At the end of the fermentation, the entire content of the SSF bottle/tray was harvested and the pretreated wood was analyzed for its acid-insoluble lignin content by the conventional two-step acid hydrolysis [6] as a reference method and by Fourier transform infrared spectroscopy. Table 1 shows the applied medium composition, fermentation set-up and fermentation duration with the corresponding AIL content for each pretreated wood sample.

2.4 Analytical methods

2.4.1 Removal of water-soluble substances

Before AIL quantification, the biomass samples were thoroughly washed to remove the lignocellulolytic enzymes and other water-soluble compounds. One rinsing cycle included the shaking of the biomass with 50 mM acetate buffer (pH 4.5) applying a solid-to-liquid ratio of 1:80 at 400 rpm for 20 min. The shaking was followed by centrifugation (Sigma 3-16KL) for 15 min at 4500 rpm and 4°C. After the removal of the supernatant, this rinsing cycle was repeated once with acetate buffer and twice with distilled water to remove the traces of acetic acid. The rinsed solid was freeze-dried (ALPHA 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH) until a constant weight was achieved and used for lignin and infrared analysis.

2.4.2 Total protein determination

Protein concentration in the rinsing liquids (2.4.1) was determined by Bradford Assay Kit (TCI Europe N.V).

2.4.3 Analysis of phenolic compounds

In the washing liquid, the detection and quantification of seven phenolic compounds, i.e. 3,4-dihydroxybenzaldehyde, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, and syringaldehyde were carried out by HPLC-UV analysis (1290 Infinity II LC system equipped with a UV-Vis diode array detector, Agilent Technologies, Inc.). The identification of each compound was based on a combination of retention time and spectral matching with external standards. The chromatographic separation was obtained on a C18 column (Aqua® 5 µm, 125 Å, 250 × 4.6 mm, Phenomenex Inc.) at 25°C. UV detection was performed at 250, 280 and 310 nm at a flow rate of 0.4 mL per minute. Combined gradient-isocratic elution was used with a mobile phase of methanol (solvent A) and 2% acetic acid in water (solvent B): 0–3 min 10% solvent A; 3–8 min a gradient to 19.3% solvent A; 8–23 min a gradient to 33.6% solvent A; 23–35 min gradient to 55% solvent A; 35–45 min a gradient to 100% solvent A; 45–60 min 100% solvent A. Prior to the HPLC analysis, all samples were filtered through a 0.2 µm polyethersulfone syringe filter.

2.4.4 Fungal biomass estimation

The ergosterol content of the fungal cell membrane was used to determine the fungal biomass in the pretreated wood before and after the rinsing cycles (Section 2.4.1). Ergosterol determination was carried out based on the study of Niemenmaa et al., 2006. For the conversion of the mass of ergosterol measured to the fungal mass, the ergosterol content of *P. chrysosporium* was determined through plate cultivation and was measured to be 7.24 ± 0.26 µg ergosterol/mg fungal biomass [4].

2.4.5 AIL determination

The acid-insoluble content of poplar wood was determined before and after pretreatment by the standard NREL protocol (NREL/TP-510-42618) [6]. Briefly, the AIL content of the samples was measured gravimetrically after two-step acid hydrolysis with sulfuric acid.

2.4.6 Milling

Before FTIR analysis, the washed and non-washed wood samples were freeze-dried (Alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH) and ground with a ball mill. Hereto, 200 mg of lyophilized sample was measured into a stainless-steel grinding jar (25 mL) together with four 10 mm and one 15 mm stainless steel grinding balls. The jar was immersed in liquid nitrogen for 30 seconds to embrittle the sample and avoid its thermal damage. Milling was carried out for 4 min at a frequency of 25 Hz in a mixer mill (MM 200, Retsch GmbH).

2.4.7 ATR-FTIR analysis

FTIR analysis was carried out on both washed (Section 2.4.1) and non-washed freeze-dried pretreated wood samples, as well as on freeze-dried *P. chrysosporium* (scraped from potato dextrose agar (PDA) plate) and on freeze-dried MnP enzymes (extract was obtained according to the study of Wittner et al., 2021). FTIR in attenuated total reflectance (ATR) mode was carried out using a Spectrum 400 spectrometer (PerkinElmer, Inc.) equipped with a Universal ATR (UATR) accessory (PerkinElmer, Inc.). Spectra were recorded from 4000 cm^{-1} to 650 cm^{-1} (i.e., the wavelength range of 2500–15385 nm) at a spectral resolution of 4 cm^{-1} with 32 co-added scans applying a contact pressure of 100 N. Samples were measured in triplicate with a Spectrum 6.3.2 software (Perkin Elmer, Inc.). Triplicate measurements were performed by placing

separate subsamples on the internal reflection element (IRE) for covering the inhomogeneity of the solid sample of biological origin.

2.4.8 Spectral data processing and multivariate analysis

All IR spectra were processed using The Unscrambler® X 10.4 (CAMO Software, Oslo, Norway) and Microsoft® Excel® 2019 (Microsoft Corp., Redmond, WA, USA) software. Standard normal variate (SNV) preprocessing was applied to reduce baseline shift caused by light scattering and by variable spectral path length [16]. After SNV normalization, principal component analysis (PCA) was performed on the washed and non-washed wood samples for dimensionality reduction and to identify outlier samples [17]. For lignin calibration, partial least squares regression (PLSR) was used which is a well-researched quantitative approach extensively referenced in the literature [18]. Prior to PLSR, the average spectra of the FTIR measurement of subsamples were calculated and were subsequently preprocessed by SNV analysis. During PLSR, the infrared spectral data are regressed against the measured acid-insoluble lignin content to find a prediction model with a high coefficient of determination (R^2) and a low root mean square error (*RMSE*). The quality of the prediction models was estimated by leave-one-out cross-validation. Therefore, during the PLSR, the performance indicators (R^2 and *RMSE*) of the models were calculated for both the calibration (C) and validation (CV) data sets.

3 Results and Discussion

3.1 Interpretation of ATR-FTIR spectra and PCA

The FTIR spectra of *P. chrysosporium,* its manganese peroxidase enzyme, and the untreated and fungus-treated poplar wood (with or without washing) are presented in Fig. 1. The wavenumber assignments of cellulose, hemicellulose, lignin and the most

significant bands assigned to the solid-state fermentation (SSF) are shown in Table 2. Since all samples were freeze-dried prior to FTIR analysis, the assumption was made that the effect of the minor amount of moisture, possibly still present after freeze-drying, is constant in all samples and neglectable.

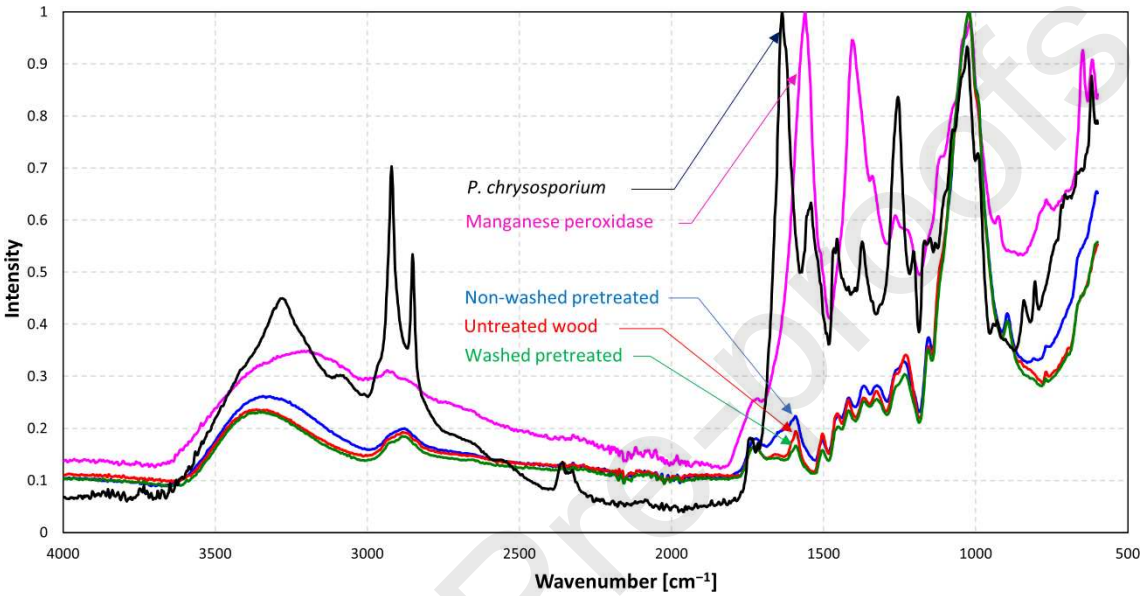


Fig. 1. FTIR characterization of (—) manganese peroxidase (MnP), (—) *P. chrysosporium*, (—) untreated poplar wood, and pretreated poplar wood (—) with and (—) without washing

Due to the complexity of woody biomass, most absorbance bands cannot be exclusively assigned to lignin but to all three wood components. The spectral region of 1700–1145 cm^{-1} provides the most information about the aromatic structure and functional groups occurring in lignin while containing the least carbohydrate-dominated bands and therefore is suitable for ATR-FTIR based lignin estimation. However, this region is also affected by the presence of extracellular enzymes such as manganese peroxidase (MnP), i.e. the main enzyme responsible for lignin degradation by *P. chrysosporium,* and by the fungus itself both absorbing at 1644 cm^{-1} (C=O stretching in the amide I peptide

[28,29]) (Fig. 1). The spectral interference of these compounds with lignin quantification was also confirmed by the principal component analysis (PCA) carried out in the region of 1700–1180 cm^{-1} on the SNV-pretreated ATR-FTIR spectra of both washed and non-washed pretreated. Since the fungal delignification starts progressively increasing after 2 weeks of pretreatment [4], the SSFs with a pretreatment time longer than 20 days (SSF23–SSF44 in Table 2), were used for the PCA analysis to obtain a good differentiation between the two sample groups (i.e., washed and non-washed). The first three principal components of the PCA analysis explained 93.64% of the variance (see Fig. S1 in supplementary materials). The first principal component (PC-1), which is responsible for 69.91% of the variance, clearly differentiates the washed and non-washed sample groups (Fig. 2).

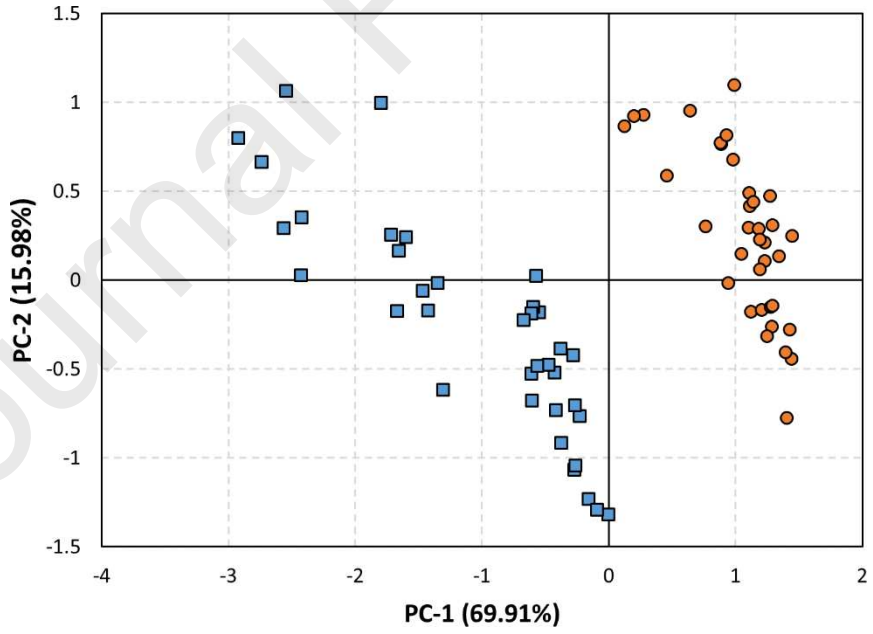


Fig. 2. PCA scores of the first two principal components for (●) washed and (■) non-washed fungus-treated poplar wood samples

The washed samples have positive scores while the non-washed SSFs own negative ones. In the loadings of PC-1, the most intense contribution is negative and found between 1700 cm^{-1} and 1500 cm^{-1} meaning that the non-washed samples own higher band intensities in this region compared to the washed samples (Fig. 3). The contribution at the spectral range of 1600–1700 cm^{-1} (Amide I vibration, arises mainly from the C=O stretching) and at 1500–1600 cm^{-1} (Amide II vibration, originates from C=N and C–N–H stretching) most probably appeared due to the enzymes secreted by *P. chrysosporium* as well as the microorganism itself both present in the non-washed samples [9,19–21].

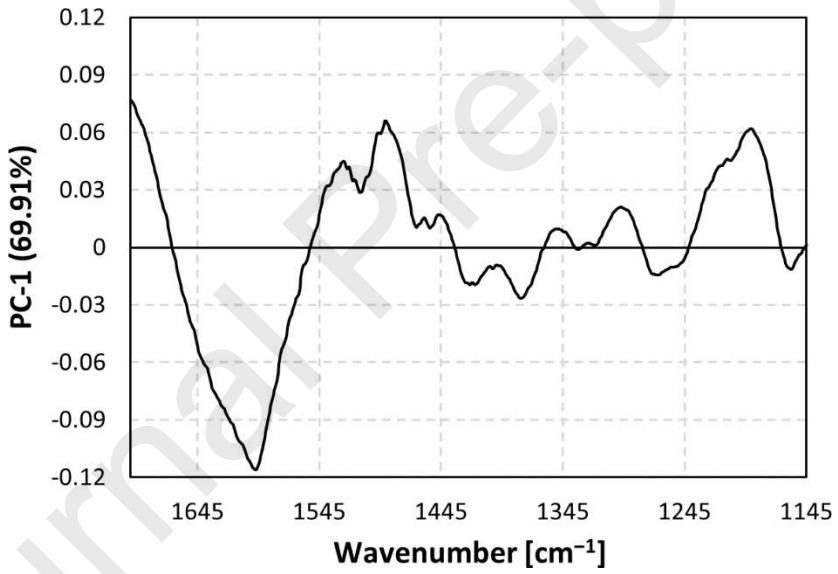


Fig. 3. PCA loading plot of the first principal component

This interpretation is supported by the ATR-FTIR spectra of the freeze-dried *P. chrysosporium* and its secreted manganese peroxidase enzyme, both absorbing at the 1500–1700 cm^{-1} region (Fig. 1). Furthermore, the removal of these compounds by washing was also confirmed by determining the protein content in the rinsing liquids and quantifying the amount of fungal biomass in the pretreated wood before and after

the rinsing procedure of the 28-day fermentations (SSF42–SSF44). The protein concentration in the washing liquids of the four consecutive washing steps showed a decreasing trend, i.e. concentrations of 9.23 ± 0.14 µg/mL, 4.31 ± 0.55 µg/mL, 1.28 ± 0.13 µg/mL and 1.02 ± 0.11 µg/mL were obtained hereby confirming the removal of lignocellulolytic enzymes produced by *P. chrysosporium*. The rinsing procedure also sufficiently decreased the amount of fungal biomass present in the pretreated wood. The initial 4.49 ± 0.09 mg fungal biomass/g dry wood was reduced to 1.10 ± 0.01 mg fungal biomass/g dry wood by the four-step rinsing procedure. However, one should consider that during solid-state fermentation the fungus deeply penetrates the wood structure and therefore cannot be quantitatively separated from it [22], thereby making a complete fungal biomass removal unfeasible. Additionally, the washing liquids were also tested for lignin-derived phenolics. However, none of the seven common phenolic compounds, i.e., 3,4-dihydroxybenzaldehyde, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, and syringaldehyde, was detected in the rinsing liquids by HPLC-UV analysis indicating the complete mineralization of lignin. Considering that in the above-discussed spectral range of 1500–1700 cm^{-1} also the important bands assigned to lignin can be found, including the aromatic skeletal vibration and C=O stretch at 1596 cm^{-1}, and the C=C stretching in the aromatic structure at 1505 cm^{-1} [23], the removal of the extracellular enzymes and the white-rot fungus by washing is essential for adequate lignin quantification.

3.2 Estimation of lignin content from ATR-FTIR

Until now, ATR-FTIR has been used only for the qualitative assessment of fungal lignin degradation [23,24] but not for the quantitative measurement of lignin in wood pretreated with fungi. Reliable lignin quantification with ATR-FTIR requires a

sufficient set of samples with great variability in lignin content and the removal of interfering proteins as discussed in Section 3.1.

In this study, a set of 45 poplar wood samples with an acid-insoluble lignin content varying from 19.9% to 27.1% were used to build the partial least squares regression (PLSR) model for lignin calibration. These samples included the raw feedstock and 44 pretreated wood samples each obtained in an individual fermentation (Table 1). PLSR models were constructed using leave-one-out cross-validation.

Most often, only certain wavenumbers of the FTIR spectra are strongly correlated to the target compound, i.e. to lignin in this study. Therefore the exclusion of the non-relevant wavenumbers can greatly improve the predictive ability of the model [25]. Therefore, first, the PLSR model was developed using the spectral range of 1800–650 cm^{-1}, since absorption bands at 3364 cm^{-1} (O–H stretching) and 2883 cm^{-1} (C–H stretching) have strong contributions from cellulose and hemicellulose [26]. By constructing our PLSR model in this range, an R^2_{CV} of 0.80 and an *RMSECV* = 0.75% were obtained for cross-validation with 5 PLSR factors. The model was further improved by selecting the spectral range of 1700–1145 cm^{-1}, i.e., the wavenumbers which provide the most information about lignin and contain the least carbohydrate dominating bands (Table 2). Using this spectral range, the obtained PLSR model required a lower number of PLSR factors (4) while it had a slightly higher R^2_{CV} value (0.81) and a decreased error (*RMSECV* = 0.73%). To further improve the predictive ability of the PLSR model, standard normal variate (SNV) normalization was applied at this spectral range before PLSR modelling. The SNV normalization improved the PLSR model in comparison with the utilization of raw spectra which was shown by a higher R^2_{CV} of 0.87 (R^2_C = 0.91

for calibration) and a lower *RMSECV* of 0.60% (*RMSEC* = 0.49%) with the same number of PLSR factors (4) (Fig. 4). These high R^2 values and low root mean square errors indicate a good prediction of lignin content considering the high variability of the heterogeneous solid-state fermentation, the varying fermentation conditions and the complexity of the reference NREL method directly affecting the prediction results.

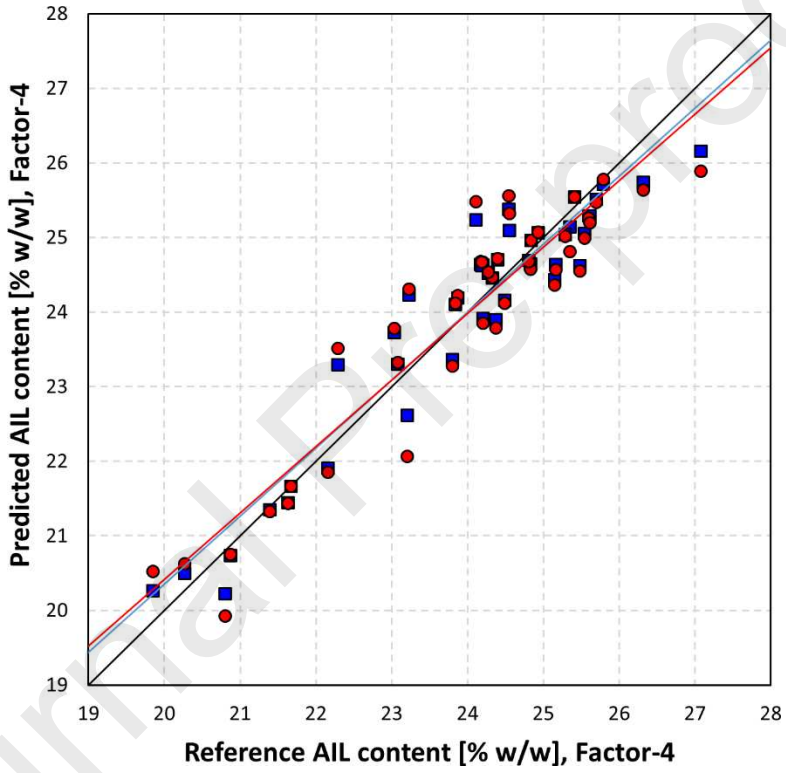


Fig. 4. The predicted vs. reference acid-insoluble lignin (AIL) values of (■) calibration and (●) validation

The interpretation of the weighted regression coefficients of PLRS factor-4 revealed that the prediction of acid-insoluble lignin content is positively correlated with the peak intensities at 1591 cm^{-1} (aromatic skeletal vibration and C=O stretch), 1504 cm^{-1} (aromatic skeletal vibration), 1456 cm^{-1} (aromatic C−H deformation), 1418 cm^{-1} (C−H

deformation), 1327 cm^{-1} (C_1–O vibration in syringyl derivatives and C–H vibration in cellulose), 1263 cm^{-1} (C–O stretch in lignin and C–O linkage in guaiacyl aromatic methoxyl groups), 1230 cm^{-1} (syringyl ring vibration, C–O stretch in lignin) [10,23,27] (Fig. 5).

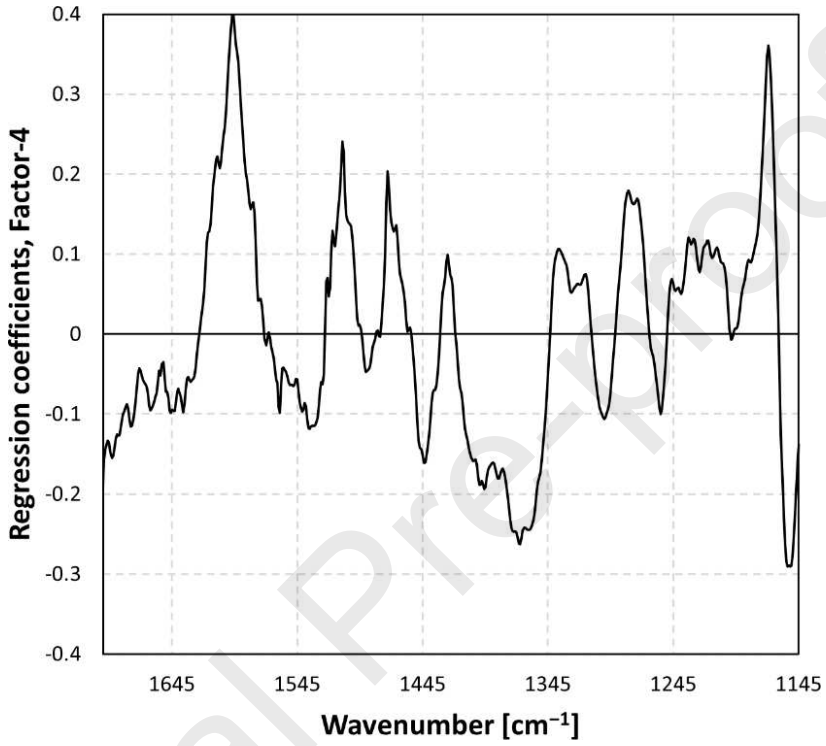


Fig. 5. Regression coefficients of PLSR factor-4 for acid-insoluble lignin determination

In this study, ATR-FTIR spectroscopy was proven to be suitable to predict AIL content in wood pretreated with white-rot fungi. A previous study using FTIR-KBr in transmission mode obtained a comparably strong correlation (R^2_{CV} of 0.91), and low error (*RMSECV* = 0.71%) while applying a higher number of PLSR factors [23]. In comparison, our proposed ATR-FTIR method does not require the preparation of a KBr pellet and therefore is an easy and high-throughput alternative to the time-consuming and exhaustive two-step hydrolysis based AIL determination in fungus-treated wood.

4 Conclusions

In this study, ATR-FTIR combined with PLSR allowed the reliable prediction of acid-insoluble lignin content in poplar wood pretreated by *P. chrysosporium*. The PLSR model was established using the spectral range of 1700−1145 cm^{-1} and had an R^2_{CV} of 0.87 and an error (*RMSECV*) of less than 1%. Compared to the conventional acid-insoluble lignin determination (> 3 h), the required sample size, sample preparation and time of lignin analysis were substantially reduced since only milling was additionally required before the spectrum acquisition with ATR-FTIR (< 10 min). The present work contributes to the faster development of the wood-based bioeconomy.

Appendix A. Supplementary data

E-supplementary data of this work can be found in the online version of the paper.

Acknowledgements

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Table 1. Fermentation conditions and the corresponding acid-insoluble lignin (AIL) content

#Sample	Set-up[a]	Medium[b]	Feedstock sterilization[c] [min]	Fermentation duration [day]	AIL content[e] [% w/w]
raw feedstock	B	-	-	-	26.32 ± 0.69
SSF1	B	Cu^{2+}+Mn^{2+}	20	28	24.54
SSF2	B	Cu^{2+}+Mn^{2+}	20	28	24.11
SSF3	B	Cu^{2+}+Mn^{2+}	20	28	24.55 ± 2.17
SSF4	B	Glucose, Cu^{2+}+Mn^{2+}	20	28	23.80 ± 0.26
SSF5	B	Glucose, Cu^{2+}+Mn^{2+}	20	28	24.37
SSF6	B	Glucose, Cu^{2+}+Mn^{2+}	20	28	23.08 ± 0.49
SSF7	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	28	19.85 ± 0.01
SSF8	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	28	20.80 ± 0.33
SSF9	B	Complex, Cu^{2+}+Mn^{2+}	20	28	20.27
SSF10	B	Complex, Cu^{2+}+Mn^{2+}	20	28	25.61 ± 0.58
SSF11	B	Complex, -	60	0[d]	25.79 ± 0.14
SSF12	B	Complex, -	60	26.8	24.20 ± 0.61
SSF13	B	Complex, Mn^{2+}	60	0 [d]	25.60 ± 0.41
SSF14	B	Complex, Mn^{2+}	60	26.8	23.20 ± 0.28
SSF15	B	Complex, Cu^{2+}	60	0 [d]	25.70 ± 0.25
SSF16	B	Complex, Cu^{2+}	60	26.8	22.29 ± 0.02
SSF17	B	Complex, Cu^{2+}+Mn^{2+}	60	0 [d]	25.29 ± 0.53
SSF18	B	Complex, Cu^{2+}+Mn^{2+}	60	26.8	21.39 ± 0.27
SSF19	T	Nitrogen, Cu^{2+}+Mn^{2+}	-	28	21.67
SSF20	T	Nitrogen, Cu^{2+}+Mn^{2+}	-	28	22.16
SSF21	T	Nitrogen, Cu^{2+}+Mn^{2+}	-	28	21.63
SSF22	T	Nitrogen, Cu^{2+}+Mn^{2+}	-	28	20.87
SSF23	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	0 [d]	27.08
SSF24	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	14	24.84 ± 0.55
SSF25	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	14	23.87 ± 0.55
SSF26	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	14	23.03 ± 0.13
SSF27	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	17	23.84 ± 0.10
SSF28	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	17	24.33 ± 0.84
SSF29	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	17	24.27 ± 0.82
SSF30	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	19	24.2

SSF31	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	19	24.17 ± 0.20
SSF32	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	19	24.81 ± 0.85
SSF33	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	21	24.19 ± 0.61
SSF34	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	21	24.93 ± 0.01
SSF35	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	21	25.35
SSF36	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	24	25.41 ± 0.20
SSF37	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	24	23.23 ± 1.27
SSF38	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	24	24.40 ± 0.22
SSF39	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	26.7	25.16 ± 0.20
SSF40	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	26.7	25.48 ± 0.08
SSF41	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	26.7	25.54 ± 0.18
SSF42	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	28	25.15
SSF43	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	28	24.49 ± 1.05
SSF44	B	Nitrogen, glucose, Cu^{2+}+Mn^{2+}	20	28	24.83

'-': not applicable

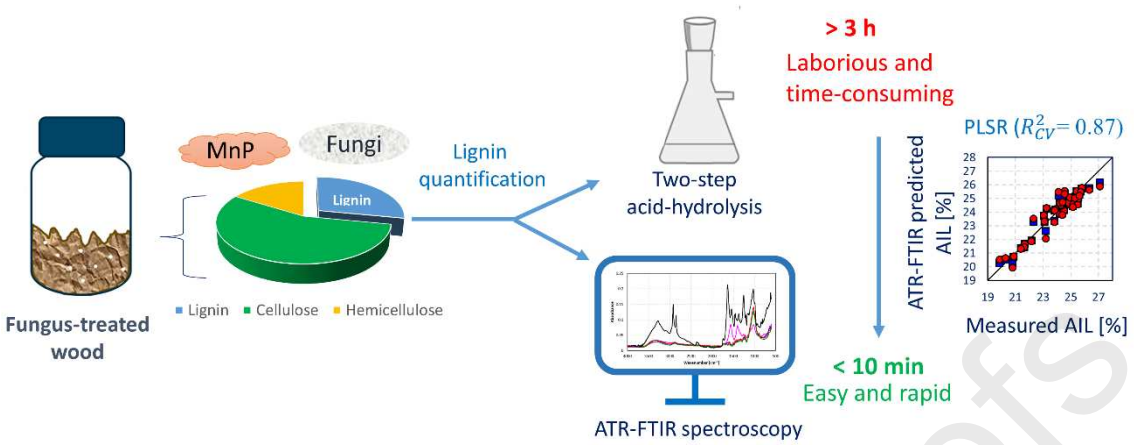
[a] B: bottle, T: tray fermentation; [b] The complex medium consists of 3 g/L $NaNO_3$, 20 g/L glucose, 0.5 g/L KCl, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L $FeSO_4 \cdot 7H_2O$, 1 g/L KH_2PO_4, 0.34 g/L veratryl alcohol, 0.1 %v/v Tween 80, 3.69 mM $CuSO_4$ and 1.41 mM $MnSO_4$. The simplified medium is composed of 3.69 mM $CuSO_4$, 1.41 mM $MnSO_4$ with or without 20 g/L glucose and/or 3 g/L $NaNO_3$. [c] Autoclaving at 121°C; [d] Fermentations immediately harvested after inoculation [e] Acid-insoluble lignin content measured once or in duplicate by the standard NREL protocol (NREL/TP-510-42618)

Table 2. ATR-FTIR peak assignment in poplar wood with and without fungal pretreatment

	Wavenumber [cm^{-1}]	Functional group	Lignocellulose/SSF related compound	Reference
Untreated poplar	895	C–H deformation	Cellulose	[10,27]
	1023	C–O stretch	Cellulose, hemicellulose	[10,27]
	1118	Aromatic skeletal vibration and C–O stretch	Lignin	[10,27]
	1156	C–O–C vibrations	Cellulose, hemicellulose	[10,27]
	1230	Syringyl ring vibrations and C–O stretch of lignin and xylan	Lignin, xylan	[10]
	1263	C–O stretch in lignin and C–O linkage in guaiacyl aromatic methoxyl groups	Lignin	[10,27]
	1327	C_1–O vibration in syringyl derivatives and C–H vibration in cellulose	Lignin, cellulose	[10,27]
	1368	C–H deformation	Cellulose, hemicellulose	[10,27]
	1418	C–H deformation	Lignin, cellulose, hemicellulose	[10,27]
	1456	C−H deformation	Lignin, cellulose, hemicellulose	[10,27]
	1504	Aromatic skeletal vibration	Lignin	[10,27]
	1591	Aromatic skeletal vibration and C=O stretch	Lignin	[10,24]
	1654	Absorbed O−H and conjugated C=O in carbonyl groups	Lignin	[10]
	1731	Unconjugated C=O stretch	Xylan (hemicellulose)	[10,24]
	2883	C−H stretching	Lignin, cellulose, hemicellulose	[10,24]
	3364	O−H stretching	Cellulose, hemicellulose	[10,27]
Pretreated poplar wood	1644	C=O stretching in the amide I peptide	Enzymes produced by *P. chrysosporium* and the fungus itself	[28,29]
	1730	C=O stretching in the amide I peptide	Enzymes produced by *P. chrysosporium* and the fungus itself	[21,28]

Highlights

1. ATR-FTIR method development for easy monitoring of fungal lignin degradation
2. Calibration data set from > 40 solid-state fermentation set-ups
3. Washing is necessary to prevent interferences of proteins and fungal biomass
4. A PLSR model with very good performance indicators was achieved
5. Greatly reduced analysis time (< 10 min) compared to wet chemistry methods (> 3 h)



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Nikolett Wittner: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization **János Slezsák:** Methodology, Formal analysis, Investigation, Writing – Review & Editing **Waut Broos**: Investigation *Jordi Geerts: Investigation* **Szilveszter Gergely**: Conceptualization, Formal analysis, Visualization, Supervision, Writing - Reviewing and Editing **Siegfried E. Vlaeminck:** Conceptualization, Supervision, Writing - Reviewing and Editing, **Iris Cornet:** Conceptualization, Data Curation, Supervision, Writing - Reviewing and Editing