"Evaluation of bacterial wood degradation by Fourier-Transform-Infrared (FTIR) measurements" *

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Abstract

Awareness of the potential role of bacterial decay of wood in water-saturated environments is relatively recent, but has led to great concern that foundation poles under historical buildings in Europe as well as waterlogged archaeological remains are under serious bacterial threat. The evaluation of the degree of degradation is essential in developing stabilisation and/or conservation strategies. Most evaluations of wood degraded by bacteria are based on physical characterization [1, 2] or microscopic observations [3-5]. The chemical composition, especially lignin content, is also a good indicator for degree of degradation [6]. These methods are known as destructive analyses and are very time and material consuming. The present study correlated changes in chemistry with the degree of microscopically detectable degradation in order to find new ways in evaluating the degree of bacterial wood degradation.

The characterization of waterlogged softwood samples by means of infrared spectroscopy reflects results of chemical analyses but a direct quantitative analysis of wood compounds by FTIR is problematic. Due the linear regression between lignin content which was determined chemically and that which was based on absorbance values of lignin in FTIR spectra a calibration curve could be drawn up. Reversed on this database it was shown that FTIR measurements are practical in evaluating the degree of bacterial degradation in softwood with the advantage of smallest amounts of sample material required, the enhanced rapidity and simplicity of this method.

Keywords: wood degradation, bacteria, decay evaluation, softwood, FTIR, Klason lignin

1. INTRODUCTION

The chemical and structural modifications which wood undergoes during burial in waterlogged conditions imply the need of an effective treatment for stabilisation *in situ* or safe conservation after discovering. However, prior to any kind of treatment, a diagnostic evaluation of the degree of degradation is essential. Till today such evaluation of the extent of decay was done by physical characteristics, like maximum water content, [1, 2] microscopic observations [3-5] or conventional chemical analyses [6, 7]. Mostly, only quick measurements of selected physical characteristics were done to evaluate the state of degradation due to the real usefulness of complete diagnostic analyses is not well connected to a practical use; current methodologies required great efforts required in terms of time and sample material.

Within this framework a reliable and quick measurement of the structural chemical components of lignocellulose materials, especially waterlogged wood, represents an important step toward a broad applicability of a systematic evaluation of the state of preservation of archaeological wood or wooden foundation piles. Infrared analyses seem to be very promising due to the relatively long experiences in their application, the fastness of execution and the smallest amounts of sample material required. However, considering the high variability in chemical structure of fossil wooden material and the presence of various other factors disturbing the interpretation of analyses, like matrix of overlapping bands in such a complex substrate like wood or considerable content of inorganic components, a research effort is required before proposing this technique as a routinely protocol for diagnostic evaluation [8]. In earlier work it could be shown that the degree of degradation evaluated per microscopy correlate with Klason lignin content of waterlogged wood samples [6, 7]. Based on a series of such preliminary evaluations via microscopy and chemical analyses performed on waterlogged softwood samples of various samples sites in Europe, the aim of this work was to develop a quantitative estimation method for chemical components of waterlogged wood by means of spectroscopic FTIR analysis.

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2. MATERIAL AND METHODS

The sample material as well as results of evaluation of the degree of bacterial degradation via microscope or chemical determined lignin content were derived from the EU-project BACPOLES (no EVK4-CT-2001-00043) of 27 sample sites in Europe. Measurements of lignin were done according to the Klason´s method (Tappi T222) [4, 5].

All FTIR measurements were carried out on oven dried sieved material (0.12 – 0.40 mm) without any special pre-treatment.

The IR spectra were recorded with a FT-IR 22 FTIR-spectrometer (Bruker, Bremen, Germany) equipped with an ATR unit (DuraSamplIRITM, SensIR Technologies, Warrington, UK) using the absorbance range from 4000 to 800 cm^{-1}. The resolution was 4 cm^{-1} and 32 scans were averaged. Background spectra were obtained without having a sample against the crystal of the ATR unit. The spectra were baseline corrected and normalized to the highest peak, so that the absorbance of the highest peak was set to 2.0. For the evaluation of the spectra only the area between wavenumbers 800 and 1800 cm^{-1} was discussed, which include the most important values of lignocellulose materials.

3. RESULTS

The microscopic evaluation of bacterial degradation were done by SHR [4] and determination of Klason lignin content were done at University of Goettingen [7] within the EU-project BACPOLES (no EVK4-CT-2001-00043). Based on these results further investigations were done by FTIR spectroscopy which results were described here exclusively.

The results of FTIR-measurements are shown exemplarily for one representative softwood sample (see Figure 1) where the outside zone is severely attacked (50 % lignin content) the middle zone show a moderate degree of attack (39 % lignin content) and the inside zone is without any sign of bacterial attack (32% lignin content).

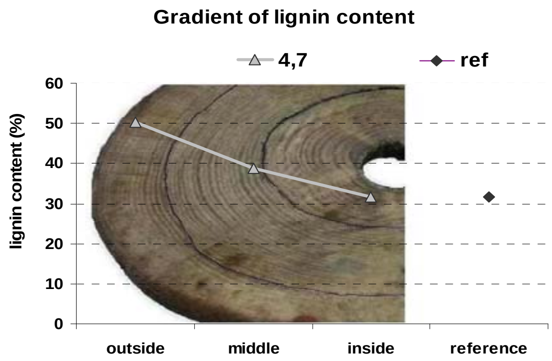

Figure 1 – Representative softwood sample with degradation gradient in three zones.

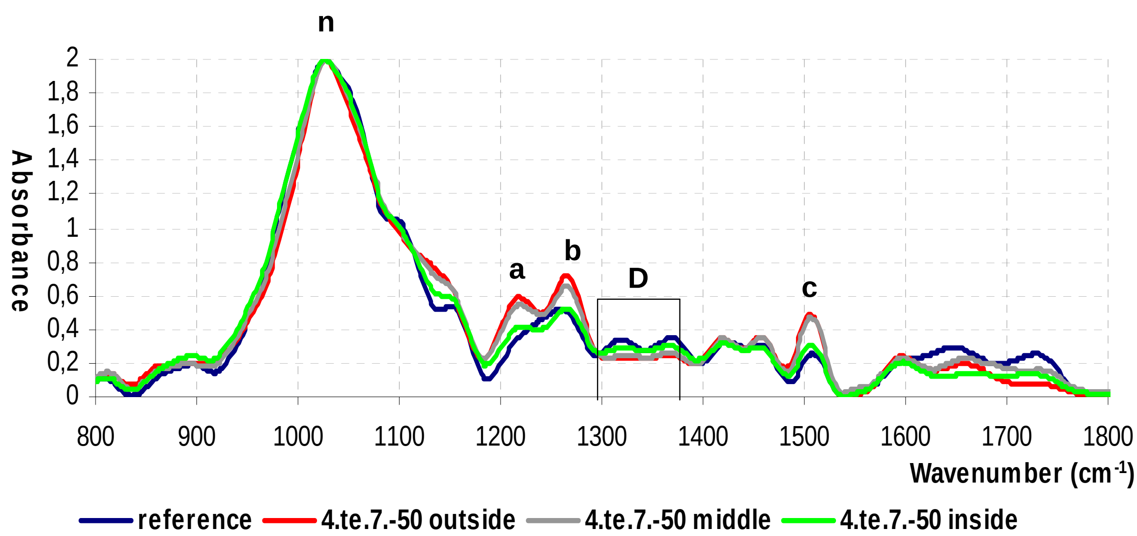

Figure 2 - FTIR spectra of a reference and three different degraded zones of one softwood sample.

In Figure 2 three peaks are higher than reference are in evidence at the wavenumbers around 1217 (a), 1265 (b) and 1505 (c) The 1217±5 cm^{-1} band is described to characterise lignin by the C-O bond of guaiacyl ring [9, 10]. The peak at 1265±5 cm^{-1} is typical for the guaiacyl ring structure in lignin [11-13] and the absorbance between the wavenumbers 1502-1506 cm^{-1} represents C=C stretching vibration in aromatic ring in lignin [12, 14-17]. Differences in the degree of degradation can be concluded from the comparison of the height of these lignin characterized peaks. The severer degraded outside zone showed higher absorbance values than the middle zone with a lower degree of degradation.

The band system between 1300 and 1380 cm^{-1} (D) derives from CH_2 bonds at 1315±5 cm^{-1}, OH groups at 1332±5 cm^{-1} and from CH bonds at 1370±5 cm^{-1} of cellulose and hemicellulose [10, 15]. That is the reason why the spectra of the degraded samples showed lower absorbance values in this range than the reference. The most degraded outside zone showed the lowest absorbance values. Even the spectra of the microscopically not degraded inside zone displayed reduced absorbance values in this range which confirm the results of chemical investigation of the holocellulose components in [9].

The results of FTIR-spectroscopy reflect the rates of chemical analysis. Due to the matrix of overlapping bands in the complex wood structure, direct quantitative analysis of several wood compounds using the FTIR spectra are problematic. But the absorbance values at wavenumbers assigned to lignin linearly correlate withchemically determined lignin contents. The highest correlation was found at the wavenumber 1505 cm^{-1} because these bands arises exclusively due to aromatic skeletal vibration of the benzene ring in lignin [10] whereas the other bands assigned to lignin derive from other functional groups. The correlation between the absorbance values at 1505 cm^{-1}, 1215 cm^{-1} and 1270 cm^{-1} of the softwood samples and their lignin content can be seen in Figure 3.

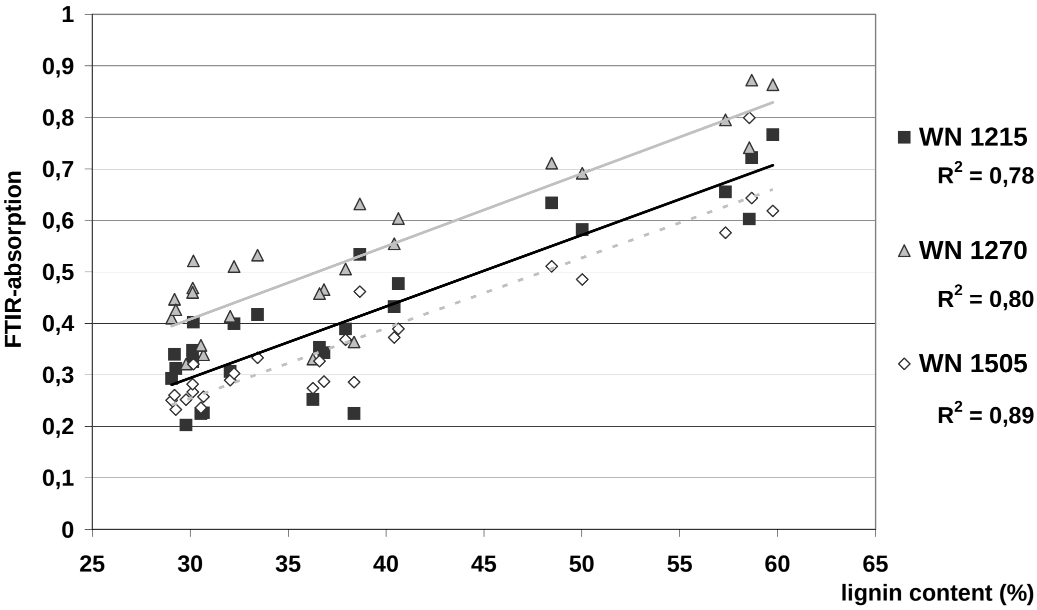

Figure 3 - Correlation between lignin content (Klason lignin) of softwood pile samples and IR absorptions at 3 representative wavenumbers (WN) [cm^{-1}] assigned to lignin.

Based on this linear correlation, determination of lignin contents, which represent the stage of degradation have been enabled for other softwood samples by FTIR measurements exclusively. In Table 1 softwood samples were evaluated in terms of the degree of bacterial degradation based on absorbance values at 1505 cm-1 and the mentioned relationships. For comparison the results of decay evaluation via microscopy done by SHR for the same samples are also presented.

Table 1 - Evaluation of some softwood samples using FTIR absorbance at 1505 cm-1 in comparison with evaluation via microscopy [4].

Sample	Absorbance at 1505 cm^{-1}	Lignin content (%) $y = 65.228x + 14.371$	Evaluation of attack based on calculated lignin content	Evaluation based on microscopic investigations by SHR
19.td.4.140	0.4426695	43.25	**severe**	**severe**
22.td.1.2420	0.3313575	35.98	**weak**	**weak**
22.td.1.6600	0.324987	35.57	**weak**	**weak**
22.td.2.2420	0.3147245	34.90	**weak**	**weak**
22.td.2.4700	0.2865405	33.06	**weak**	**weak**
24.ta.1	0.551838	50.37	**severe**	**severe**
24.ta.2	0.6029455	53.70	**severe**	**severe**
24.ta.3	0.515601	48.00	**severe**	**moderate-severe**

4. DISCUSSION AND CONCLUSIONS

The results obtained suggest that it is possible to build models to assess the lignin content of waterlogged archaeological wood by means of FTIR measurements using simple linear regression analysis. Concerning [8] some precautions should, however, to be considered in effectively applying this method, due for instance to the influence of inorganic fillers. Used samples showed ash contents between 0.5 and 10% [9] which seem to be unproblematic in FTIR analyses. But in archaeological wood ash contents up to 69% [8] are possible which can influence the chemical lignin determination and the FTIR-spectra.

Further analysis including more samples of different wood species in combination with multivariate regression techniques will be tested to assess lignin content or other components relevant for the degradation of archaeological wood.

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