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Use of NIR spectroscopy to monitor substrate biodegradation lignocellulosics by *Pleurotus*

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Abstract

Understand the process of biodegradation of lignocellulosic material as its chemical composition is key properties for enhancing the cultivation of edible mushrooms, which seek in lignin and other carbon source carbohydrates. The purpose of this study was to monitor polysaccharide and lignin degradation caused by *Pleurotus ostreatoroseus* on different agroindustrial substrates using near infrared spectroscopy (NIR). Sawdust substrates of Marupá and Cajuí wood, banana pseudostem fibers, enriched with bran of cereals were formulated (WB and MWB). Of the 12 formulations elaborated, eight were submitted to biodegradation of *P. ostreatoroseus* over a period of 49 days (Residual substrate), and four formulations maintained *in natura* (Initial substrates), where each treatment was formed by 10 replicates totaling n = 120. Spectra were obtained from the substrates before and after cultivation in FT-NIR system in the region between 10,000 and 4,000 cm^{-1} and the index degradation of lignocellulosic compounds was determined (LDI and PDI). Through Fourier transform near infrared spectroscopy was it is possible to observe the degradation of polysaccharides and lignin in the substrates grown with the fungus *P. ostreatoroseus*. In the residual substrate Marupá+WB-POSP was the highest lignin degradation index (LDI = 9.26%). While whereas for the PDI (Polysaccharide degradation index), the highest degradation (7.12%) was observed in the residual substrate Cajuí+MWB-POAM. Through analysis of the absorption bands, it was possible to observe the degradation of structures/bonds characteristics of lignocellulosic residues. The NIR model for degradation of lignocellulosic compounds was more effective in predicting "lignin degradation" in the spectral range 6,350-7,308 cm^{-1}.
Keywords: agro-industrial waste, degradation, lignin, near infrared

Introduction

The fungus *Pleurotus* has been studied in many parts of the world for its value gastronomy, ability to colonize and degrade a wide variety of waste lignocellulosics. Relatively short cycle compared to other mushroom genera as well as harness rustic environments for production. The consumption of edible mushrooms has been growing significantly due to recognition of its high nutritional value and increased supply, making the product most popular and affordable (Sales-Campos et a., 2017; Garuba et al., 2017).

The use of agricultural waste has become a sustainable practice, as recyclability as well as waste disposal, thus alternative for environmental balance. Wood waste substrates, agricultural and agroindustrial crops are used to grow edible fungi. The main constituents of agro-industrial substrates are polysaccharides (cellulose and hemicellulose), lignin and extractives. Its quantification is primordial for technological characterization of end products, since several authors have attributed the growth velocity mycelial and the quality of the mushroom (Menezes et al., 2010; Zhang & Su, 2014).

Near infrared spectroscopy (NIR) has been widely used in several areas, since it is a fast technique, does not require preparation, reagents and does not generate waste. NIR spectra have absorption bands attributed to overtones and combination bands generated mainly by the vibration of groups C-H, O-H and C=O specific grouping chemical structures. This non-destructive tool can be used to estimate sample concentrations or monitor structural changes during chemical, physical and chemical processes biological. In addition to multivariate calibration, it is possible to discriminate between the samples and with this one can explain certain relationships of the process of biodegradation (Nascimento et al., 2012; Wei et al., 2017).

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Biomonitoring studies of the degradative process of wood and substrates industry has been using infrared spectroscopy as a tool for elucidation of these processes. Most biodegradation studies are based on mass loss suffered by the specimens, and wet analysis (Monteiro et al., 2014; Gümüştaş & Bayram, 2018). Xiaoyu et al. (2007) evaluating the biological activity of basidiomycetes, confirmed lignin degradation through NIR spectroscopy. Krongtaew et al. (2010b) evaluated agricultural substrates using Fourier transform spectroscopy (FT-NIR) and obtained standard analytical models that served as references to attribute physical and chemical changes to the substrates. While Segura et al. (2008) investigated the influence of *Ganoderma* and *Lentinus* strains on lignin degradation by FT-IR.

Understand the chemical composition and its relationship in the substrate before and after cultivation in mushroom production is a determining factor. Therefore, studies of chemical characterization of the raw materials, in the case of this study, waste agribusiness. The objective of this study was to monitor the degradation of polysaccharides and lignin caused by *Pleurotus ostreatoroseus* on different substrates industry using spectroscopy NIR.

Material and Methods

Substrates formulated from mixtures of wood sawdust (Marupá and Cajuí) and banana cv Caipira variety, enriched with cereal bran (Wheat bran; Mixture of wheat bran, corn and rice) in the proportions of 40:40:20 were used in the study (**Figure 1**). From these substrates were cultivated *Pleurotus ostreatoroseus* from Manaus-AM (POAM) and São Paulo – SP (POSP) strain within 49 days.

After cultivation, the substrates were autoclaved and oven-dried at 100 °C, grinding (Willey mill) and sieving (40, 60 and 80 mesh) in a sieve shaker (Ro-Tap/Testing sieve shaker), and at the end the fractions were stored in glass vial for further analysis. **Table 1** shows the substrates and residuals used in the study.

Table 1. Quantity and composition of lignocellulosic substrates used in the study

Woods	Marupá		Cajuí	
Enrichment	WB	MWB	WB	MWB
Initial (*in natura*) IS	10	10	10	10
Residual Manaus-AM RS *P. ostreatoroseus* POAM	10	10	10	10
Residual São Paulo-SP RS *P. ostreatoroseus* POSP	10	10	10	10

WB = Wheat bran; MWB = Mixture of wheat bran, corn and rice

The experimental design adopted in this step was distributed in a factorial scheme 2 X 1 X 2 X 2 X 10. Two raw materials (Marupá and Cajuí) were used, one residue banana, two enrichment (WB and MWB), two fungal lines (Manaus-AM and São Paulo-SP) and 10 substrates, corresponding to eight formulations submitted to biodegradation (Residual substrate), each with 10 repetitions, and four formulations kept in natura (Initial substrates), also with 10 replicates totaling n = 120.

Near infrared spectra were obtained from the initial substrates and post-cultivation (40 mesh particle size in climate-controlled environment, T = 20 ± 2 °C and RH = 65 ± 5%) in an FT-NIR Antaris II Thermo Scientific system. The samples were placed to glass cuvettes (3.0 x 5.0 cm) in a rotary system to obtain the using RESULT™ analysis software in the region between 10,000 and 4,000 cm^{-1} with 8 cm^{-1} resolution 96 scans were accumulated for each sample, and replicas mean spectra were calculated in the OMNIC® package.

Figure 1. General scheme of the preparation of the studied substrates: A - banana pseudostem; B - Sawdust; C and D - Preparation of substrate; E - Flask used in the experiment, with fully colonized substrate.

The region of 6,916-6,913 cm^{-1} was used as a pattern of group vibration phenolic hydroxyl (OH-stretch) characteristic of lignin. While for polysaccharides (cellulose and hemicellulose) the selected region was 5,290-5,210 cm^{-1} (OH- stretch) according to Krongtaew et al. (2010b).

The degradation of both lignin and polysaccharides was calculated based on the absorbance intensity of substrates before and after mushroom cultivation (Segura et al., 2008). The Lignin degradation index equals $\%LDI_{band\ lignin} = \lambda IS - \lambda RS / \lambda IS \times 100$ (λIS = Absorbance of initial substrate; λRS = Absorbance of residual substrate), while the Polysaccharide degradation index equals $\% PDI_{band\ polysaccharides} = \lambda IS - \lambda RS / \lambda IS \times 100$.

The values of the results obtained for LDI and PDI were submitted to analysis of variance (ANOVA), with the aid of the PAST 2.17c software (demo version), to verify if there was statistical difference between the substrates. For data that differed statistically, that is, when the value of F was significant ($a = 0.05$), the Tukey test with 5% significance level.

Results and Discussion

Through Fourier Transform Near Infrared Spectroscopy (FT-NIR) it was possible to observe the degradation of lignin in substrates cultivated with *Pleurotus ostreatoroseus*, since the characteristic band of the hydroxyl grouping phenolic (OH bond = 6,916-6,913 cm^{-1}) presented variation in the absorbance of initial -IS and residuals substrates -RS (**Figure 2**). In analyzing the results of the intensities of post-cultivation absorbances (RS), it was found that the highest lignin degradation (LDI) was observed in the RS Marupá+WB-POSP (9.26%), Cajuí+MWB-POAM (8.33%) and Cajuí+WB-POAM (7.55%). While the lowest was observed in Cajuí+WB-POSP (0.10%) and Marupá+MWB-POAM (0.98%) according to Table 2.

Highest LDI detected in Marupá+WB-POSP residual substrate may be associated greater reduction in absorbance in the aromatic OH characteristic band which was 0.540A in the IS and reduced to 490A in the RS by *P. ostreatoroseus* fungus. The substrates cultivated with Manaus - AM lineage (POAM) presented on average higher lignin biodegradation (~ 16%) in relation to São Paulo - SP (POSP) strain, regardless of substrate composition.

The characteristic bands of the polysaccharides that make up cellulose and hemicelluloses adopted in infrared spectroscopy are located in the range between 5,290-5,210 cm^{-1} with OH- bond vibration of various sugars according to Krongtaew et al. (2010a). In substrates grown with *P. ostreatoroseus* (POAM and POSP) the higher values of polysaccharide degradation - PDI were observed in the Cajuí+MWB-POAM (7.12%) and Marupá+WB-POSP (7.09%) RS (**Table 2**). When compared to the degradation of polysaccharides in the substrates by the two fungal strains (Manaus and São Paulo) it can be observed that a difference of 14% largest for the Manaus-AM strain.

NIR spectroscopy proves to be a fast and accurate tool for determining lignocellulosic compounds, where absorption bands are spectra from the vibrational combination of the 1st overtone of C-O, O-H, C-H and N-H (Ma et al., 2018). The characteristic band of lignin is attributed to the phenolic hydroxyl group (OH-). The change in absorption in this spectral range indicates that biodegradation led to increase in phenolic groups, supposedly caused by the degradation of ether-O-4 between hydrolysis of methoxy groups (Mitsui et al., 2008).

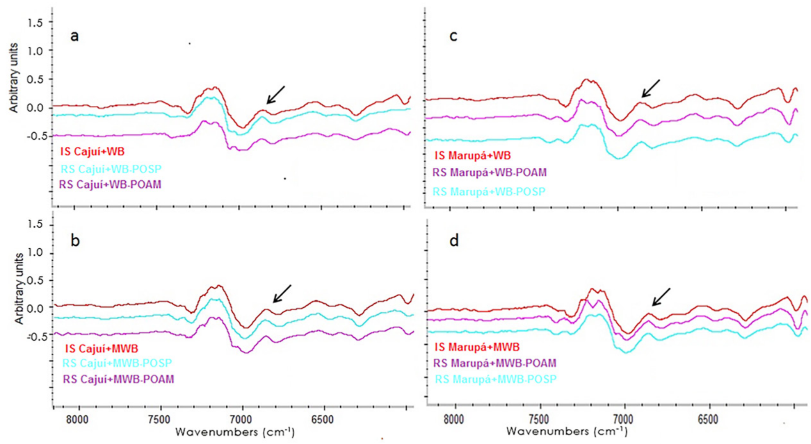

Figure 2. NIR spectra of initial (IS) and residual (RS-POAM and RS-POSP) substrates with 2nd derivative treatment (Norris filter) highlighted band characteristics of OH - lignin bonds (6,916-6,913 cm^{-1}): a – IS and RS Cajuí+WB; b - IS and RS Cajuí +MWB; c – IS and RS Marupá+WB; d - IS and RS Marupá+MWB

Table 2. Lignin degradation and Polysaccharides in substrates grown with *Pleurotus ostreatoroseus*

Woods	Enrichment	Lineage *Pleurotus*	LDI (%)	PDI (%)
Marupá	WB	Manaus-AM	5.56 c	3.94 b
		São Paulo–SP	9.26 a	7.09 a
	MWB	Manaus-AM	0.98 d	0.50 d
		São Paulo–SP	4.90 c	3.88 b
Cajuí	WB	Manaus-AM	7.55 b	4.03 b
		São Paulo–SP	0.10 d	0.10 d
	MWB	Manaus-AM	8,33 a	7.12 a
		São Paulo–SP	4.63 c	2.36 c
Mean Manaus-AM			5.61	3.90
Mean São Paulo–SP			4.72	3.36

LDI = Lignin degradation index; PDI = Polysaccharide degradation index; Numbers shown are equivalent to the average values of residual substrates. Means followed by the same letter do not differ statistically by the Tukey test at a probability level of 5%

Table 3. Characteristic bands for lignocellulosic components

Chemical component	Link/Vibration*	Band location (cm^{-1})
Lignin	CH stretch 1° overtone	6,916, 6,060, 5,980 and 5,479
	C=O stretch	6,420 and 6,372
	OH stretch 1° overtone	6,913
Polysaccharides	CH stretch 1° overtone/ Glucose arabino	6,488
	C=O stretch 2° overtone/ Xilose	6,952
	OH stretch 1° overtone/ Glucose/xilose	7,142, 6,757, 6,024, 5,290 and 5,208

*Krongtaew et al. (2010a); Schwanninger et al. (2011); Ma et al. (2018)

NIR spectrum changes of substrates are interpreted as changes quantitative and/or qualitative in terms of reducing absorption in a single wavelength which may indicate a removal of aromatic structures thus providing carbon and energy source for fungal development *Pleurotus*. Dashtban et al. (2010) report that certain basidiomycetes besides degrading the cellulose and hemicelluloses also produce decomposition of lignin.

Wang et al. (2019), working with *P. ostreatus* inoculated cotton stalk reported preferential degradation of lignin with a ~10% decrease after 30 days of incubation. While, Segura et al. (2008) treating the grass *Pennisetum purpureum* x *Pennisetum typhoides* for the same period in the process of selective solid state fermentation with *Ganoderma* strains detected about 70% of aromatic compounds relative to aliphatic compounds.

In the RS Marupá+MWB-POAM and Cajuí+WB-POSP, we observed low LDI (<1%), giving the idea that the phenolic complex present in the substrates, was not degraded by the tested microorganism, implying that there has been an increase in this substance. Schmidt et al. (2003) studying lignin degradation hay for Brachiaria by *P. ostreatus*, found behavior similar to that observed in the research, that is, there was an increase of lignin in the substrates. In this case of substrates Marupá and Cajuí, for these samples the spectral study, in the range defined in the work, was not efficient to detect degradation in the specified band. Krongtaew et al. (2010a) states the existence of other characteristic bands in the aromatic CH bond vibration for lignin at 5,980, 5,800 and 5,479 cm^{-1} (**Table 3**), which could explain more efficacy the lignin degradation in these substrates.

The residual substrates Marupá+MWB-POAM and Cajuí+WB-POSP were observed. PDI ≤ 0.50%, when the 5,290-5,201 cm^{-1} band was used. This result is considered anomalous, since in practice fungi of the genus *Pleurotus* are excellent polysaccharide degraders. An explanation for this discrepancy according to Nascimento et al. (2012) is associated with the defined spectral region (5,290 cm^{-1}) in this same range can be found water molecule (H-O-H) and so the absorbance read can be combined with the molecular vibration of water and not with the polysaccharide structure. Further characteristic bands should be investigated later, which may validate the degradation of the substrates in question.

In agricultural residues the most abundant polysaccharides are cellulose and hemicelluloses. The first is formed entirely by β-D glucose sugar, and for hemicellulose is a complex of D-xylose, L-arabinose, D-galactose, D-mannose as well as to a lesser extent L-rhamnose, L-fucose and other sugars neutral (Carvalho et al., 2009; Carvalho et al., 2013).

Complete degradation of polysaccharides depends on different enzymes such as cellulase, xylanases among others. Basidiomycetes usually produce large amounts of enzymes that degrade lignocellulolytic substrates, e.g., such as the high secreting fungi *Phanerochaete chrysosporium* and *Coriolus versicolor* a-glucuronidase levels, while *Cuninghamella subvermispora* produces xylanase in wood sawdust (Carvalho et al., 2009; Menezes et al., 2010).

Pleurotus ostreatus, *Pleurotus sajor-caju* and *Trametes versicolor* are also producers lignocellulolytic enzymes, where the endoxylanase found in these microorganisms results in the production of xylanases with rigorous specificity in different substrates (Milagres et al., 2005). Understand the degradation process of compounds such as lignin and polysaccharides in substrates grown by *Pleurotus* may explain growth strategies/routes of these organisms in mushroom cultivars.

Conclusions

Near infrared spectroscopy (NIR) has potential as analytical tool for monitoring the degradation of *Pleurotus ostreatoroseus*. Through the analysis of NIR absorption bands, it was possible to observe the degradation of characteristic lignin structures/bonds on different substrates.

The data obtained in this work indicate a possible indication of spectroscopy in the to assist in elucidation of biotransformation processes of chemical components of agro-industrial substrates, quickly in relation to the conventional techniques. This study indicates new advances in the near future in the area of biotechnology about *Pleurotus* production, with process improvement and control of quality.

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