

Article

Nanostructural Changes Correlated to Decay Resistance of Chemically Modified Wood Fibers

Nayomi Z. Plaza ^{1,*} , Sai Venkatesh Pingali ² and Rebecca E. Ibach ^{1,*} 

¹ Forest Products Laboratory, Forest Service, United States Department of Agriculture, Madison, WI 53726, USA

² Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA; pingalis@ornl.gov

* Correspondence: nayomi.plazarodriguez@usda.gov (N.Z.P.); rebecca.e.ibach@usda.gov (R.E.I.)

Abstract: Reactive chemical modifications have been shown to impart decay resistance to wood. These modifications change hydroxyl availability, water uptake, surface energy, and the nanostructure of wood. Because fungal action occurs on the micro and nano scale, further investigation into the nanostructure may lead to better strategies to prevent fungal decay. The aim of this article is to introduce our findings using small angle neutron scattering (SANS) to probe the effects of chemical modifications on the nanostructure of wood fibers. Southern pine wood fiber samples were chemically modified to various weight percentage gains (WPG) using propylene oxide (PO), butylene oxide (BO), or acetic anhydride (AA). After modification, the samples were water leached for two weeks to remove any unreacted reagents, homopolymers or by-products and then the equilibrium moisture content (EMC) was determined. Laboratory soil-block-decay evaluations against the brown rot fungus *Gloeophyllum trabeum* were performed to determine weight loss and decay resistance of the modifications. To assist in understanding the mechanism behind fungal decay resistance, SANS was used to study samples that were fully immersed in deuterium oxide (D₂O). These measurements revealed that modifying the fibers led to differences in the swollen wood nanostructure compared to unmodified wood fibers. Moreover, the modifications led to differences in the nanoscale features observed in samples that were exposed to brown rot fungal attack compared to unmodified wood fibers and solid wood blocks modified with alkylene oxides.

Keywords: wood fiber; wood modification; brown rot; fungal decay; SANS; acetylation; propylene oxide; butylene oxide



Citation: Plaza, N.Z.; Pingali, S.V.; Ibach, R.E. Nanostructural Changes Correlated to Decay Resistance of Chemically Modified Wood Fibers. *Fibers* **2022**, *10*, 40. <https://doi.org/10.3390/fib10050040>

Academic Editors: Vincenzo Fiore and Carlo Santulli

Received: 4 March 2022

Accepted: 24 April 2022

Published: 28 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Solid wood and products containing wood fibers, like other biological materials, are susceptible to deterioration when used outdoors [1]. Covalently attaching compounds to the hydroxyl groups in wood (solid or fibers) provides increased protection against decay from brown rot, for instance, without leaching out toxic chemicals into the environment [2]. The mechanisms by which these chemical modifications provide improved properties, particularly fungal decay resistance, is still an ongoing area of research. Most research indicates that lowering the moisture content of the end product is important for the decreased susceptibility of modified wood to wood-decay fungi [3–6]. Moreover, recent studies have shown that chemical modifications can lead to nanostructural changes [7,8] and alter the nanoscale moisture distribution inside wood cell walls [9–12], which in turn likely has an impact on the susceptibility of wood nanostructure to biodegradation. An improved fundamental understanding of how modifications change the nanostructure of solid wood and fibers and how these changes are correlated to the moisture content would accelerate the development of new protection treatments. Probing these nanostructural changes (ranging from 1 to over 100 nm) can be difficult due to the inherently low contrast between the different lignocellulosic polymers. Small-angle neutron scattering (SANS) is uniquely suited to access this information with minimal sample preparation, and contrast

can be easily increased by introducing heavy water (D₂O) into the wood cells either via moisture uptake [13,14] or full sample immersion [7,8,15].

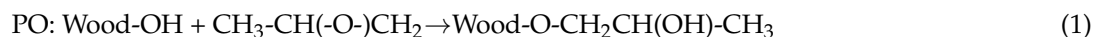
While studying the chemical modification of wood fibers has the potential of providing new insights due to the increased number of surfaces available for modification, much of the research on chemical modification of wood has focused on solid wood. Previous studies with modified solid wood found a correlation between the equilibrium moisture content (EMC) and decay resistance for wood modified with some chemicals [16]. For solid wood modified with butylene oxide (BO) and acetic anhydride (AA), the decay resistance increased as the EMC of the wood decreased [17]. By contrast, in PO-modified wood, the decay resistance increased with increasing weight percentage gain (WPG), but the EMC did not decrease. More recently, SANS studies have revealed that solid wood chemically modified for decay resistance with high levels of alkylene oxides preserved the microfibril structure from fungal decay and reduced the swelling of the microfibrils. [11]. While SANS has been used to monitor the nanostructural changes that occur during the dilute acid pretreatment of switchgrass [18] and steam-heat treating of aspen wood chips [19], the changes caused by modification of wood fibers have not been explored yet.

Here, we studied the effects of modifying wood fibers with three different chemicals—two alkylene oxides, BO and PO, and one anhydride, acetic anhydride (AA)—on the fungal decay and the equilibrium moisture content of the modified fibers. Furthermore, using SANS we found that different chemicals altered differently the wood fiber nanostructure (i.e., spatial features within 1 to 100 nm), and that all effective chemical modifications were able to preserve the modified nanostructure from fungal decay to different extents, indicating different nanostructural pathways to achieve fungal decay resistance.

2. Materials and Methods

Butylene oxide (BO) and acetic anhydride (AA) were obtained from Eastman Chemical Company (Kingsport, TN, USA), while propylene oxide (PO) and triethylamine (TEA) were obtained from Sigma-Aldrich Chemical Company (Milwaukee, WI, USA). The southern pine wood fiber (*Pinus* spp.) was obtained from Templen Forest Products Corporation (Lubbock, TX, USA). The fiber was produced using pulp chips from the paper mill that were hard cooked in a batch digester for 6 min at 180 psi steam. The heated chips were then run through a Bauer Single Refiner to produce the fiber.

Chemical modifications: Southern pine fiber was screened with a fine sieve (stainless steel mesh of 200 × 200) to remove any fine particles. The fibers were then washed with reverse osmosis (RO) water, oven dried at 105 °C for 24 h in a forced draft oven and weighed prior to reaction with BO, PO, or AA. The reactions between the wood hydroxyl groups and PO, BO and AA are described in Equations (1)–(3), respectively:



BO and PO reactions were performed in a stainless steel reaction vessel (Parr Instrument Company, Moline, IL, USA). The vessel was loaded with fibers (about 30 g), the alkylene oxide (either BO or PO) and the catalyst (TEA) (95:5 (v:v)), and then flushed with dry nitrogen. The temperature was raised gradually to 110 °C or 120 °C and then the vessel was pressurized to 150 psi and held for various times [20,21]. The reaction times (up to 6 h) were measured from the point of reaching the reaction temperature until the vessel was put in cold water to stop the reaction. The treating solution was drained off. Chemically modified fibers were air-dried under a fume hood overnight prior to oven drying at 105 °C for 24 h. This process was repeated several times to produce a total of 120 g of modified fibers of each modification level. The weight percent gains (WPG) were calculated from the average oven-dried weights of the individual batches (3–4 replicates). For acetylation, the fiber was dipped in AA for 1 min. Excess solution was drained for 5 min. The fiber

was placed in a one-liter glass reactor that was heated with an oil bath at 120–125 °C from 22 min up to 240 min. The modified fiber was oven dried at 105 °C for 24 h and the WPG was calculated. A portion of fibers (about 30 g) from each level of modification were water leached for 14 days [22] and WPG was calculated. Acetyl content was determined on both the leached and unleached unmodified and acetylated fibers (AF) using anion-exchange, high-performance liquid chromatography with suppressed conductivity detection [17].

Equilibrium moisture content (EMC): EMC of unmodified and modified fiber was determined by placing 1 g of oven-dried fiber in a constant humidity room at 90% relative humidity (RH) and 27 °C. The fiber samples were wrapped in netting material to allow even airflow around the samples without loss of fibers (Supplementary Figure S1a). After 14 days, samples were reweighed until stable, and the EMC was determined. Six replicates of each treatment were run and averaged.

Brown rot fungal exposure: A modified ASTM D 1413 standard soil block test was performed on fiber samples [23] (Supplementary Figure S1b). Five soil bottles each of unmodified controls and BO, PO, or AA-modified samples of southern pine fiber (0.5 g) samples were exposed to the brown rot fungus *Gloeophyllum trabeum* (Pers (Murril) 1908 MAD 617) (USDA-NRS-FMHC, Forest Products Laboratory, Madison, WI, USA) in an environmental chamber set at 26.7 °C and 70% RH. Two soil bottles each with no fungus were run to monitor leaching of any unreacted reagents, homopolymers, or by-products. The fiber weight loss of the unmodified controls was monitored weekly. When 50% weight loss was reached with the unmodified fiber controls (at 10 weeks), all fiber samples were removed from the test and transferred to small glass weighing bottles, where the fibers were air dried overnight and then oven dried for 24 h. The extent of decay was determined as oven dry weight loss (WL). To determine the WL solely caused by the brown rot exposure, the no-fungus WL was subtracted from all other WL measurements to remove any contributions caused by the leaching of any chemicals during the 10-week test. This test was performed on unleached and water-leached samples.

Small angle neutron scattering (SANS): A subset of samples with different WPGs and WLs were measured using SANS. Selected WPGs and WLs of the measured fibers were comparable to those WPG and WL values of solid wood modified with PO and BO, previously reported by Ibach et al. 2022 [11]. A collection of wood fibers weighing about 0.03 g total was placed inside the Titanium cells with 1mm path length (Supplementary Figure S1c), and the cell was filled with D₂O. These fiber samples were soaked in D₂O inside the cell for over 12 h; the cell was re-filled with D₂O as needed and any air bubbles were removed. All SANS measurements were performed at the Bio-SANS instrument in the Cold Guide Hall of the High Flux Isotope Reactor (HFIR) facility of the Oak Ridge National Laboratory (ORNL). For these measurements, the neutron incident wavelength was 6 Å, with a relative wavelength spread of 13.2%. To access a wide dynamic q-range of 0.003–0.85 Å^{−1}, a single dual-detector configuration was used with the main detector at 15.5 m, and the curved west wing detector (that is fixed at a radius of 1.13 m) was rotated to 1.4°. The detector patterns were normalized by monitor counts and corrected for dark current, pixel sensitivity, solid angle, and background. Then, using the ORNL neutron facility developed drt-sans reduction script provided by the instrument scientist, SANS data were reduced and azimuthally averaged isotropically to produce 1D SANS profiles. The isotropic scattering profiles were fitted using a Unified Fit model with three structural levels to model changes in the low-q ($q < 0.01 \text{ Å}^{-1}$), mid-q ($0.01\text{--}0.08 \text{ Å}^{-1}$) and high-q ($0.08\text{--}0.5 \text{ Å}^{-1}$) regions as conducted previously for analysis of SANS data from lignocellulosic biomass [18,24]. A power-law exponent, P, and/or a characteristic dimension, R_g, were extracted for each structural level [25,26]. A detector artifact was observed around $q = 0.5 \text{ Å}^{-1}$; thus, data beyond this value were not included in the fitting range. All analysis was performed using the Unified Fit tool in the Irena macro [27] in Igor Pro 8 (Wavemetrics Portland, OR, USA).

3. Results

3.1. Chemical Modification, Decay and EMC

The reaction times of BO were run up to a maximum of 6 h, whereas the reaction times of PO were only run to a maximum of 60 min to achieve weight percentage gain (WPG) values around 20 and 25 WPG, respectively (Tables 1 and 2). There was only a small amount of weight loss after water leaching (less than 2%) compared to a previous study on solid wood (between 2 to 8%) [11]. The higher reaction temperature of 120 °C provided only slightly higher WPG compared to the 110 °C samples for both BO and PO. The reaction times of AA were run up to 4 h to achieve a maximum of ~15WPG and ~16–17% acetyl content (Table 3). After two weeks of water leaching, only negligible weight loss and acetyl loss was observed for the AA modified samples.

Table 1. Weight percent gain of BO modified wood fiber before and after water leaching.

Butylene Oxide (5% TEA, 150 psi)			
Reaction Temperature	Reaction Time (min/h)	Unleached (WPG)	Water Leached (WPG)
110 °C	0 min	0	−3.3
110 °C	20 min	6.5	6.1
110 °C	1 h	11.8	11.1
110 °C	2 h	17.9	17.0
110 °C	4 h	20.7	19.6
120 °C	6 h	20.9	19.5

Table 2. Weight percent gain of PO modified wood fiber before and after water leaching.

Propylene Oxide (5% TEA, 150 psi)			
Reaction Temperature	Reaction Time (min/h)	Unleached (WPG)	Water Leached (WPG)
110 °C	0	0	−2.1
110 °C	5 min	7.5	7.0
110 °C	15 min	15.3	14.2
110 °C	30 min	21.0	19.6
110 °C	60 min	24.9	23.6
120 °C	60 min	25.7	24.1

Table 3. Weight percent gain and acetyl content of AA modified wood fiber before and after water leaching.

Acetic Anhydride					
	Reaction Time (min/h)	Unleached (WPG)	Acetyl (%)	Water Leached (WPG)	Acetyl (%)
Fiber	0	0	1.0	−3.9	1.0
1 min dip	22 min	4.5	7.5	4.1	6.4
5 min drain	40 min	8.8	11.9	8.6	12.0
120–125 °C	2 h	13.4	15.3	12.8	15.5
oil bath	4 h	14.8	16.3	14.3	17.0

When determining the fungal decay resistance of lignocelluloses with the soil block test [28], samples with <10% weight loss are considered a success (highly resistant). Weight losses in excess of 11% but less than 24% are considered resistant and weight losses between 25% and 44% are considered moderately resistant. Over 44% weight loss is a failure (not resistant). Modification with BO was highly resistant (<1% WL) at 18 WPG for both the leached and unleached samples. The PO modified fibers were highly resistant at 15 WPG

(3% WL) for the unleached and 21 WPG (1.7% WL) for the leached samples. The AA-modified fiber was highly resistant in arresting decay at 9% WPG (12% acetyl) for the unleached (5.9% WL) and 13% WPG (15.5% acetyl) for the leached (2.7% WL) samples.

Figure 1 shows the weight loss and the EMC of the water-leached samples for each WPG evaluated. All modifications showed fungal decay resistance with increasing WPG. BO and AA showed a correlation between decay weight loss and EMC; in other words, as the weight loss from decay decreases, so does the EMC, whereas the PO did not show a decrease in EMC as the decay weight loss decreased.

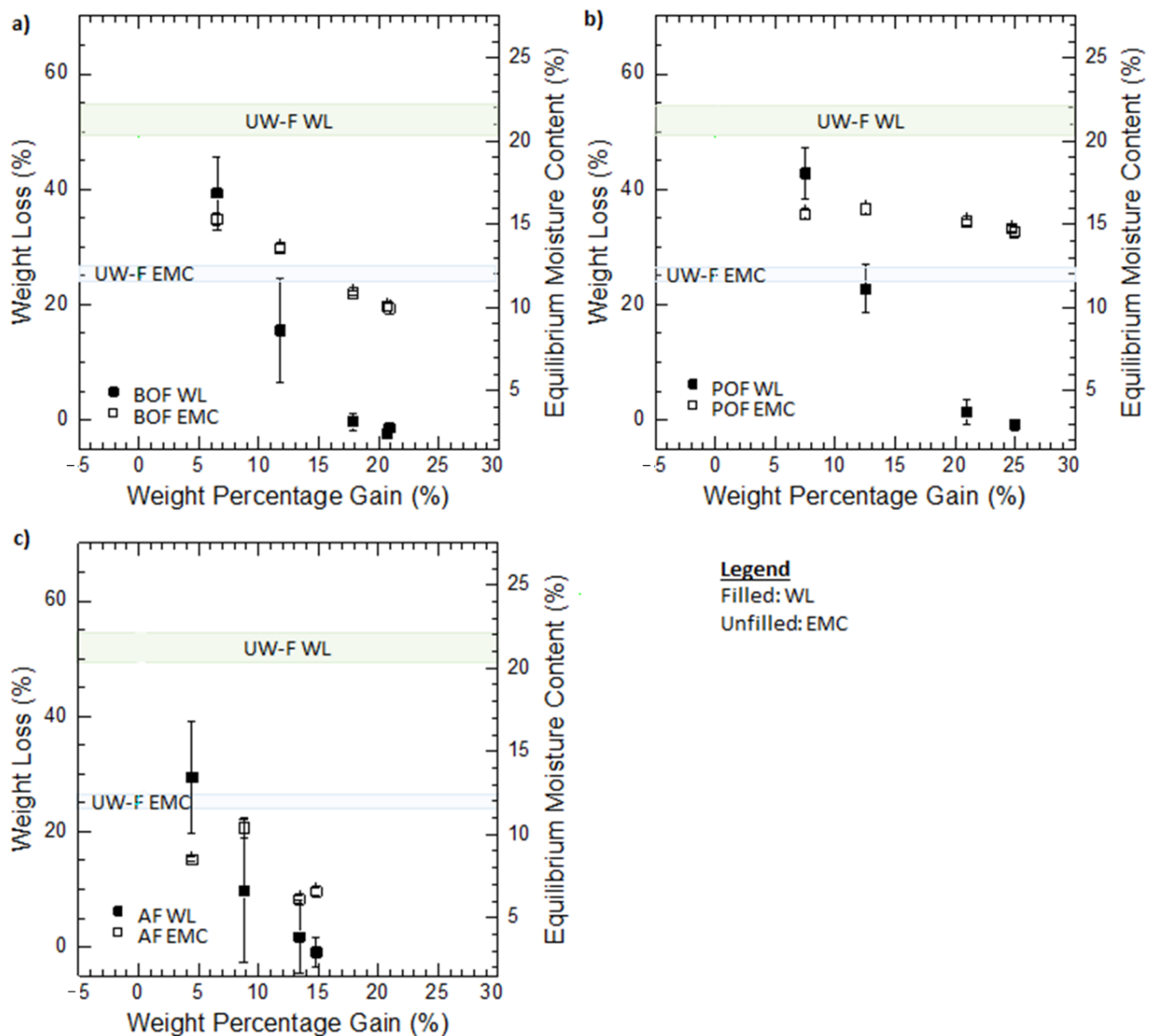


Figure 1. Effects of two different alkylene oxides and one anhydride modification on the weight loss (WL, left-y axis) and equilibrium moisture content (EMC, y-right axis) at 90% RH and 27 °C for wood fibers modified with (a) butylene oxide (BOF), and (b) propylene oxide (POF), (c) acetic anhydride (AF). EMC measurements were performed with six replicates, WPG were calculated from triplicates, and WL measurements had 5 replicates. Data points correspond to the mean values and the error bars are the standard deviations.

To further understand the mechanism of fungal decay resistance in the modified wood fibers, SANS experiments were performed on a subset of wood fibers with and without

brown rot exposure. Details with regards to the modification and decay levels of these samples are listed in Table 4.

Table 4. Description of samples studied using SANS.

Sample Description	Modification Level WPG (%)	Decay Level WL (%)
Unmodified Wood Fiber (UWF)	0	-
UWF exposed to brown rot exposure (UWF-BRE)	0	54.9
Wood fiber lightly modified with butylene oxide (BOF7)	6.5	-
Wood fiber modified with butylene oxide (BOF21)	20.9	-
BOF2 exposed to brown rot (BOF21-BRE)	20.9	−0.6
Wood fiber lightly modified with propylene oxide (POF7)	7.5	-
Wood fiber modified with propylene oxide (POF21)	21.0	-
POF7 exposed to brown rot (POF7-BRE)	7.5	43.5
POF21 exposed to brown rot (POF21-BRE)	21.0	1.7
Acetylated wood fiber (AF15)	14.8	-
AF exposed to brown rot (AF15-BRE)	14.8	0.4

3.2. Nanostructure of Chemically Modified Wood Fibers

The scattering length density (SLD), which is a measure of how strong neutrons scatter by a molecule, of heavy water (D_2O) is much larger ($6.3 \times 10^{-6} \text{ \AA}^{-2}$) than the SLD of the wood polymers even after chemical modification ($1.87 \times 10^{-6} \text{ \AA}^{-2}$ for cellulose vs. $1.1\text{--}1.6 \times 10^{-6} \text{ \AA}^{-2}$ for a modified wood polymer). Thus, when unmodified wood fibers or modified fibers are immersed in D_2O , the source of contrast giving rise to isotropic scattering profiles is the same: water-accessible regions vs. non-water-accessible regions inside the wood fibers. The scattering profiles of all the fibers measured in this study consist of three structural levels, namely, the low-q, mid-q, and high-q regions (Figure 2). Scattering from smooth surfaces dominates below $q = 0.01 \text{ \AA}^{-1}$ and are responsible for a sharp increase in the power-law exponent of the low-q scattering. Modifying the fibers leads to distinct differences in the mid-q ($0.01 \text{ \AA}^{-1} < q < 0.08 \text{ \AA}^{-1}$) and high-q ($q > 0.08 \text{ \AA}^{-1}$) regions. The size of lignin agglomerates and/or the cross-section of bundles of cellulose elementary fibrils (i.e., microfibrils) can contribute to the scattering in the mid-q region, whereas the diameter of the individual elementary fibrils contributes more strongly to the high-q region. Thus, the similarities between the profiles of the heavily modified fibers indicate that at this level the modification is altering the wood fiber nanostructure similarly. For all modified fibers, we observed an increase in the mid-q region combined with a decrease in the high-q region. This indicates that there is less water going inside the cellulose microfibrils, and thus the scattering contribution of the individual elementary fibrils is reduced. At the lower levels of modification, we observe differences between BO- and PO-modified wood fibers that are not noticeable at the higher levels. BOF7 is more closely templating the unmodified wood nanostructure (Figure 2a), whereas the nanostructure of the POF7 is similar to that of the POF21 sample (Figure 2b). For the AF15 (Figure 2c), we observe an increase in the mid-q scattering region similar to the PO samples, but the scattering of the high-q region is not as strongly reduced as in other modified samples.

By fitting the SANS data with a Unified Fit model with three structural levels, we can quantify the differences observed in the mid-q and high-q regions in terms of their associated power law and/or characteristic dimension R_g , which are listed in Table 5. Chemical modification did not change the low-q power law scattering exponent, which was close to ~ 4 for all samples, indicating scatterings from relatively smooth surfaces of large objects are dominating this region. Thus, the power law exponent was fixed at four for subsequent analysis for most samples. In the mid-q region, the cross-section of the microfibrils, whose mean diameter is about 20 nm [15,29–31], as well as hydrophobic nanodomains in the wood polymer matrix can contribute to the scattering intensity. The unmodified fibers and BO-modified fibers only showed power law scattering with exponents ranging between ~ 1 to ~ 1.5 . This behavior is likely caused by the strong contribution of the scattering from the long cellulose fibrils. For PO- and AA-modified fibers, the most

pronounced change is the emergence of a new characteristic length scale in the mid- q region that is shown as a shoulder around $q \sim 0.025 \text{ \AA}^{-1}$ in Figure 2b,c. The size of this feature is larger in the AF sample than in the POF samples, and the underlying power law is also different between these two modifications. These differences may be in part attributed to the differences observed in the high- q region. For the AF sample, the scattering from the elementary fibrils is stronger and their R_g is larger than the one observed for unmodified fiber (Supplementary Figures S1 and S2). Conversely, for PO fibers, the scattering contribution from the elementary fibrils is reduced, which increases the uncertainty in the measured R_g value (Supplementary Figure S3).

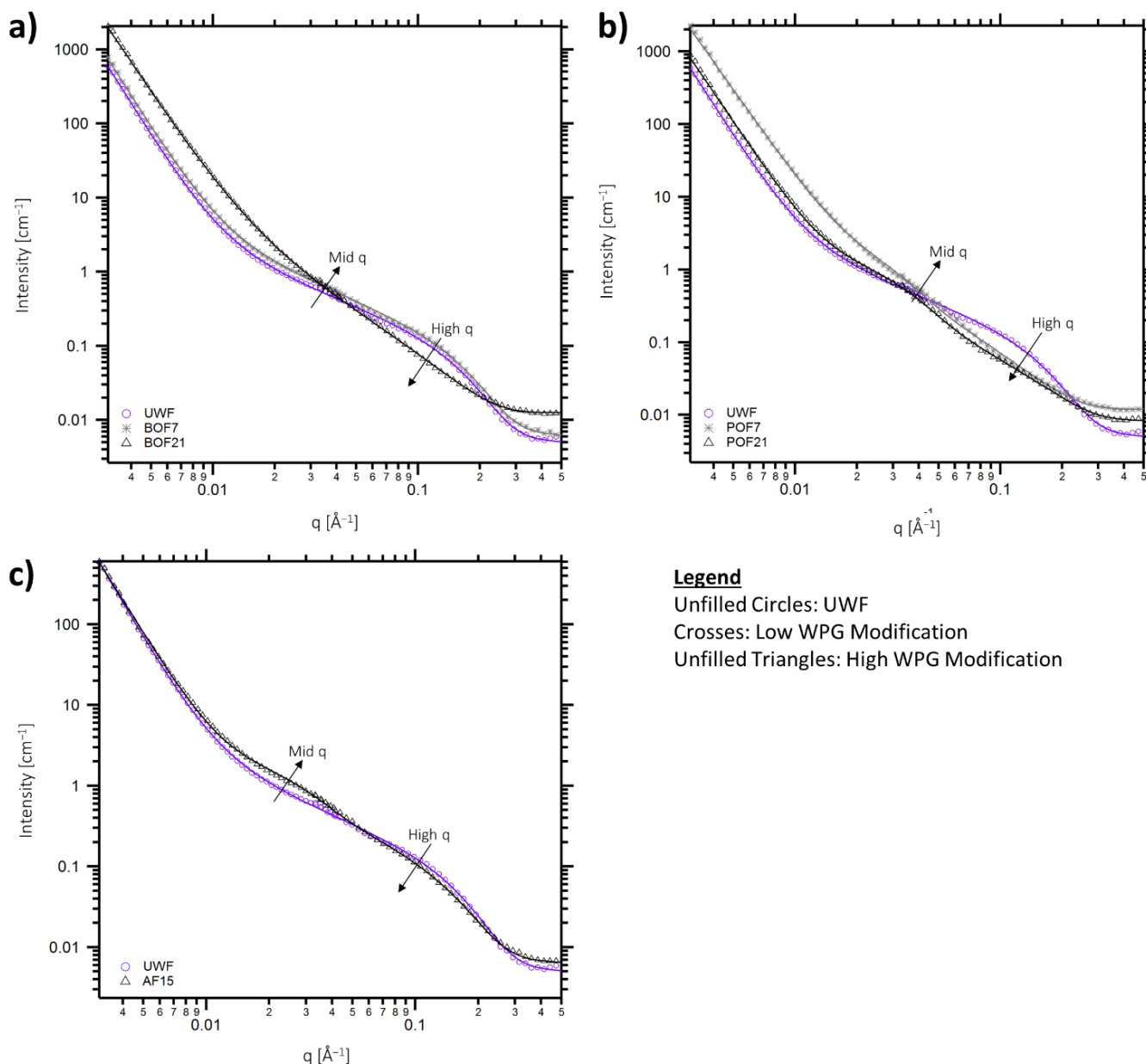


Figure 2. Effects of chemical modification on the SANS profiles obtained from wood fibers modified with (a) butylene oxide, (b) propylene oxide and (c) acetic anhydride. Data is shown as open symbols, and overlaid lines correspond to model fits. The arrows indicate the effects of increasing WPG on the mid- q and high- q scattering regions.

Table 5. Effects of modification on the scattering parameters. Uncertainties for each parameter are included in parenthesis.

Sample	Low-q $q < 0.01 \text{ \AA}^{-1}$	Mid-q $0.01 \text{ \AA}^{-1} < q < 0.08 \text{ \AA}^{-1}$		High-q $q > 0.08 \text{ \AA}^{-1}$
	P_1	P_2	$R_g \text{ (\AA)}$	$R_g \text{ (\AA)}$
UWF	4	1.01 (0.02)	-	11.5 (1.3)
BOF7	4	0.95 (0.06)	-	11.6 (1.4)
BOF21	4	1.5 (0.06)	-	11.4 (1)
POF7	3.7 (0.02)	2.1 (0.3)	48.3 (2.9)	9 (4.1)
POF21	4	2.9 (0.4)	52.2 (2.6)	11.9 (2)
AF15	4	1.8 (0.7)	65.4 (4.6)	13.6 (2.4)

3.3. Effects of Brown Rot Exposure on Nanostructure of Chemically Modified Wood Fibers

Once the unmodified wood fibers have been exposed to brown rot fungi for 10 weeks, they become discolored and more brittle; however, there are no changes in the nanostructure due to the exposure, as reflected by the almost identical scattering features between UWF and UWF-BRE (Figure 3a). The scattering profiles from BRE-modified wood fibers show differences in the mid-q and high-q regions compared to the UWF-BRE. These differences indicate that the brown rot exposure interacted differently with the modified wood polymers and consequently gave rise to different nanostructural features. For instance, for BOF21-BRE there is a clear mid-q scattering shoulder that was not observed in the BOF21 sample. The presence of a shoulder in the profile indicates that there is a new particle/agglomerate with a characteristic length scale contributing to the scattering. The increased mid-q scattering shoulder (around $q \sim 0.25 \text{ \AA}^{-1}$) has been previously attributed to the formation of repolymerized lignin agglomerates in brown-rot-exposed biomass, whereas for POF21-BRE, the scattering intensity in the high-q region increases and resembles more the scattering from unmodified wood. For AF15-BRE, the two shoulder features become more distinct and both shift towards higher q (Figure 3d). This shift indicates that the characteristic length scales associated with the shoulders are smaller for the AF15-BRE compared to the AF15 sample.

The structural parameters obtained from fitting the scattering profiles to a Unified Fit model with three structural levels are listed in Table 6. No differences were observed at the low q ($q < 0.01$), and preliminary fits indicated that the low-q power law exponent was ~ 4 for all samples, indicative of scattering from relatively smooth surfaces. Given the short range over which the low-q power law dominates and the lack of differences between samples, for most samples the power law exponent of this level was fixed at 4 for the subsequent uncertainty analysis. All modified fiber samples showed increased power law exponents and a shoulder in the mid-q region with an R_g of about $50 \text{ \AA}$ for all samples, regardless of the chemical used. Differences in the mid-q region between UWF-BRE and the modified fibers indicate that the modification changes how the brown rot exposure affects the wood fibers' nanostructure. In the high-q region, there was an increase in the R_g for unmodified fibers and fibers modified with PO compared to samples that had not been exposed to brown rot. By contrast, the high-q R_g in BO fibers was unchanged after exposure, indicating that the cross-section of the elementary fibrils was protected from decay. For the AF15 fibers, the R_g decreased in size post-exposure and became comparable to the values observed for UWF.

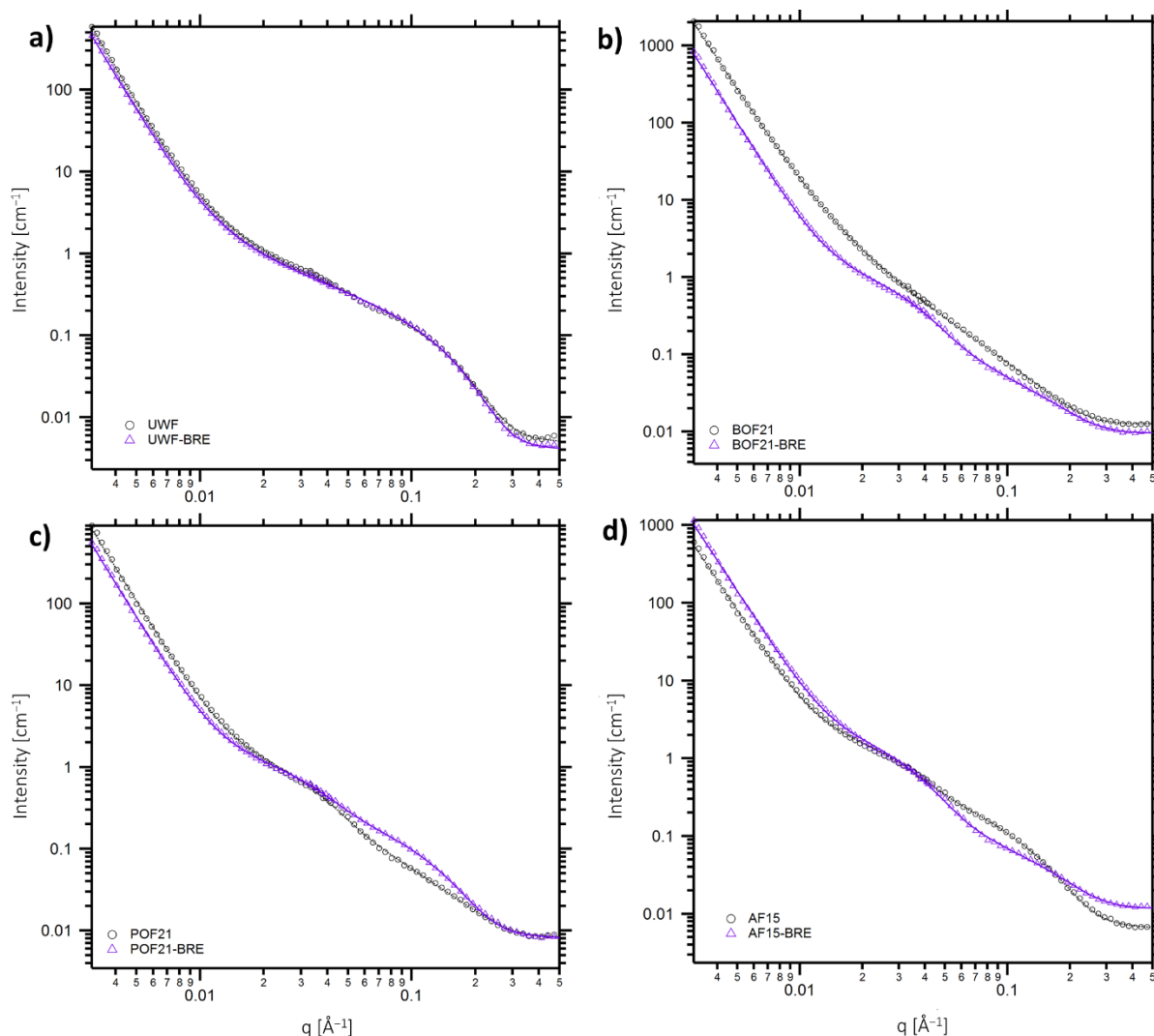


Figure 3. Effects of brown rot exposure on the SANS profiles from (a) unmodified wood fibers and fibers modified with (b) butylene oxide, (c) propylene oxide and (d) acetic anhydride. Open symbols and overlaid lines correspond to SANS data and model fits, respectively.

Table 6. Effects of brown rot exposure on the scattering parameters. Uncertainties for each parameter are included in parenthesis.

Sample	Low-q $q < 0.01 \text{ Å}^{-1}$	Mid-q $0.01 \text{ Å}^{-1} < q < 0.08 \text{ Å}^{-1}$		High-q $q > 0.08 \text{ Å}^{-1}$
	P_1	P_2	$R_g \text{ (Å)}$	$R_g \text{ (Å)}$
UWF-BRE	4	0.95 (0.02)	-	13.5 (0.8)
BOF21-BRE	4 (0.07)	3.36 (0.4)	50.4 (1.5)	11.8 (1.3)
POF7-BRE	4 (0.05)	2.8 (0.1)	55.8 (2.2)	10.3 (3)
POF21-BRE	4	2.2 (0.1)	50.5 (3.5)	15.4 (1.6)
AF15-BRE	4	3 (0.4)	54.9 (3.4)	11 (0.6)

4. Discussion

Combining SANS with EMC and fungal resistance analysis revealed that effective chemical modifications can arrest decay by imparting different changes to the wood fiber nanostructure. The differences observed between the different chemical modifications suggest that the chemicals interact with different wood polymers to impart decay resistance,

which leads to changes in the effective R_g of the elementary fibrils before and after brown rot exposure and new hydrophobic domains whose R_g is in the range of 6–8 nm. Some of these nanostructural features are comparable to those previously observed in decay-resistant, chemically modified solid wood [11], despite the fact that the wood fibers modified in this study are likely not representative of those found in unmodified wood, due to differences in their processing history.

The process used to produce the wood fibers in this study likely led to coalescence of individual elementary fibrils, which in turn increased the high- q R_g compared to unmodified/native wood. Thermal treatments have been shown to increase cellulose crystallite size in cellulosic systems [32–34] and even lead to increased R_g , due to the coalescence of elementary fibrils in steam-treated aspen [19]. Hydrothermal treatments of aspen have even led to the formation of lignin agglomerates; however, in this work we did not observe this effect, probably due to the relatively short time of hydrothermal treatment used to produce the fibers. Regardless of this pre-modification effect, after a 10-week exposure to brown rot, the R_g of the elementary fibrils increased from 11.5 to 13.6, which agrees with previously reported data showing that as the decay by *G. trabeum* progresses, the wood nanostructure opens up, due to the exposure, and eventually the cellulose amorphous surfaces are eroded [24,35,36].

Heavily modifying solid wood with over 15WPG of alkylene oxides such as epoxy-butene, propylene oxide and butylene oxide resulted in similar mid- q shoulder features [11] as those observed in PO and AA modified fibers. For modified solid wood, this mid- q shoulder was previously attributed to scattering from new hydrophobic domains that are formed due to the modification, which could include: bundles of elementary fibrils that are inaccessible to water as well as modified wood polymer nanodomains in the matrix. The similarities between PO-modified solid wood and fiber indicate that the source of scattering is similar, regardless of any potential effects of the pre-processing of the fibers. Considering that PO and AA have been reported to be more reactive with lignin [21,37], it is likely that their interaction with lignin led to the increased mid- q scattering. In the context of the wood cell wall nanostructure, this would indicate that both PO and AA are likely interacting with the lignin-rich matrix, where the cellulose microfibrils are embedded with different degrees of efficacy. For AA, which is also reactive with hemicellulose, the interaction between AA and the wood polymers leads to the emergence of a mid- q shoulder while preserving the high- q scattering contribution from the elementary fibrils, which is reduced in PO-modified fibers. This would indicate that modification with AA keeps water outside the cellulose microfibrils, whereas PO does not. For BO fibers, there is no mid- q shoulder observed, even though a mid- q feature was observed for BO-solid wood. This would indicate that the source of the mid- q shoulder was different between BO and PO. In solid wood, BO lowered both the EMC and the microfibril swelling with increasing WPG; thus, it is conceivable that the size of the bundles of inaccessible elementary fibrils was contributing strongly to the mid- q shoulder, whereas in the BO fibers, this contribution would have been much weaker because fiber processing likely increased the microfibril size.

Although all fiber modifications were considered effective at higher WPGs, they imparted decay resistance by modifying the nanostructural degradation differently. This clearly indicates that there are various pathways to attain fungal decay resistance that are likely influenced by the wood polymers spatial architecture as well as any preferential interactions between the chemicals and the wood polymers. While the spatial architecture of the wood polymers in the wood cell walls is still being elucidated, most recent evidence suggests that hemicelluloses such as xylan and glucomannans are closely interacting with the cellulose elementary fibrils [38–40], whereas lignin is mostly found outside the cellulose microfibrils, whose cross-section is about 20 nm [15,29–31]. The lignin in the matrix is thought to form nanodomains or agglomerates [41,42] with a broad size distribution (below 200 nm) that are subject to deformation and can be repolymerized by the brown rot exposure [6,43]. The R_g of these lignin agglomerates ranges from 6 nm to over 13 nm, and its emergence has been observed due to biorefinery-relevant pre-treatments [19,44,45], BR

exposure, and/or treatments meant to mimic biological degradation [24,36]. The formation of repolymerized lignin aggregates increases with BR exposure time (from 0 to 42 days), and any remaining fungal presence (i.e., enzymes, mycelia, etc.) did not contribute to the scattering data [24]. It should be noted that these repolymerized lignin agglomerates may not be recognizable as lignin that is initially found in the wood cell walls [46,47]. Since AA and BO were both able to protect the individual elementary fibrils from degradation and the R_g remained unaltered (compared to UWF) after exposure, this would suggest that these chemicals are likely interacting with the hemicelluloses and/or the amorphous celluloses between elementary fibrils. On the other hand, PO, which had about 2% WL due to BRE, did not protect the elementary fibrils, and the R_g increased following the BR exposure. The differences observed between the different chemicals studied suggest that AA and PO interact with the lignin-rich matrix and BO does not. The differences in the mid-q power law scattering behavior between AA and PO suggest that their interaction with the lignin-rich matrix is different. For AA, which lowered the EMC of the samples with increasing WPG, the modification likely led to acetylated microfibrils that are inaccessible to water and thus contribute to the scattering in this region. The modification with PO, which did not lower the EMC with increasing WPG, seems to have led to modified nanodomains that are not effective at preventing water from entering the microfibrils. Thus, the scattering from these modified nanodomains is likely contributing more strongly to the scattering in this region. Our data show that the most decay-resistant modifications protect the elementary fibrils and also lower the moisture content of the modified fibers much lower than 16% [48–51], which has been previously proposed to be necessary to hinder diffusion through the wood cell walls and prevent the onset of fungal decay.

5. Conclusions

Chemical modification of fiber is faster and less water leachable than with the solid wood, even at the slightly lower reaction temperature. These attributes give it great potential as a fiber source for composite materials that will be exposed to adverse environments. Combining SANS with EMC and fungal-resistance analysis revealed common nanostructural features in decay-resistant wood fibers that are like those previously observed in chemically modified solid wood. Chemical modification of fibers with BO and AA showed a correlation between the EMC and fungal resistance, which followed the moisture-exclusion mechanism. However, SANS revealed that these two modifications changed the wood fiber nanostructure differently. AA modified the wood fiber nanostructure similarly to PO, which showed no correlation between the EMC and fungal resistance. Moreover, while all modifications were able to arrest decay at high WPGs, SANS revealed differences in the degradation of the modified wood nanostructures. BO and AA were effective at lowering the MC and protected the elementary fibrils from being depolymerized by the BR exposure, whereas PO did not protect the elementary fibrils from degradation by *G. trabeum* even when the modification provided sufficient decay resistance. Our findings indicate that there are two different nanoscale mechanisms to impart decay resistance to the wood nanostructure: directly protecting the elementary fibrils, which correlates with wood fibers' MC, or modifying the matrix outside the microfibrils, which did not correlate with the fibers' MC. New protection chemistries could be developed by drawing inspiration from these mechanisms. Future work using SANS to characterize how different fungi (i.e., soft rot, white rot, other brown rots) degrade wood would be valuable.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/fib10050040/s1>. Figure S1: (a) Pine wood fibers (1g) wrapped in netting material to determine the EMC at 90% RH and 27 °C. (b) Pine wood fibers (0.5 g) of unmodified control, acetylated unleached (14.8 WPG) and acetylated leached (14.3 WPG) samples during exposure to the brown rot fungus *G. trabeum* in the soil block test. (c) Pine wood fibers inside the titanium cell filled with D₂O for SANS measurements. Figure S2: SANS profile from unmodified wood fibers (UWF) showing the overall fit (overlaid black line), as well as the individual contributions of the background low-q, mid-q, and high-q terms. Figure S3: SANS profile from acetylated wood fibers (AF15) showing the

overall fit (overlaid black line), as well as the individual contributions of the background low-q, mid-q, and high-q terms. Figure S4: SANS profile from wood fibers modified with propylene oxide (POF21) showing the overall fit (overlaid black line), as well as the individual contributions of the background low-q, mid-q, and high-q terms.

Author Contributions: Conceptualization, N.Z.P. and R.E.I.; methodology, N.Z.P., R.E.I. and S.V.P.; formal analysis, S.V.P. and R.E.I.; investigation, N.Z.P., R.E.I. and S.V.P.; resources, S.V.P.; data curation, N.Z.P. and R.E.I.; writing—original draft preparation, N.Z.P.; writing—review and editing, N.Z.P., R.E.I. and S.V.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available upon request.

Acknowledgments: A portion of this work performed acknowledges the support of the Genomic Science Program, Office of Biological and Environmental Research (OBER), U.S. Department of Energy (DOE), under Contract FWP ERKP752. The SANS studies on Bio-SANS (IPTS-21873.1) were supported by the OBER funded Center for Structural Molecular Biology (CSMB) under Contract FWP ERKP291, using the High Flux Isotope Reactor supported by the Basic Energy Sciences, Department of Energy. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy. This manuscript has been coauthored by UT-Battelle, LLC, under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The authors would like to acknowledge assistance from Beom-Goo Lee and Barbara Hogan.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Feist, W.C. Outdoor Wood Weathering and Protection. In *Archaeological Wood: Properties, Chemistry and Preservation. Advances in Chemistry Series 225, Proceedings of 196th Meeting of the American Chemical Society, Los Angeles, CA, USA, 25–28 September 1988*; Rowell, R.M., Barbour, R.J., Eds.; American Chemical Society: Washington, DC, USA, 1989.
2. Rowell, R.M.; Rebecca, E.; Nilsson, T. Chapter 8 Wood Durability and Stability Without Toxicity. In *Sustainable Development in the Forest Products Industry*; Universidade Fernando Pessoa: Porto, Portugal, 2010; pp. 181–208.
3. Ringman, R.; Beck, G.; Pilgård, A. The Importance of Moisture for Brown Rot Degradation of Modified Wood: A Critical Discussion. *Forests* **2019**, *10*, 522. [\[CrossRef\]](#)
4. Ringman, R.; Pilgård, A.; Brischke, C.; Richter, K. Mode of action of brown rot decay resistance in modified wood: A review. *Holzforschung* **2014**, *68*, 239–246. [\[CrossRef\]](#)
5. Thybring, E.E.; Kymäläinen, M.; Rautkari, L. Moisture in modified wood and its relevance for fungal decay. *IForest* **2018**, *11*, 418–422. [\[CrossRef\]](#)
6. Goodell, B.; Winandy, J.E.; Morrell, J.J. Fungal Degradation of Wood: Emerging Data, New Insights and Changing Perceptions. *Coatings* **2020**, *10*, 1210. [\[CrossRef\]](#)
7. Chen, P.; Li, Y.; Nishiyama, Y.; Pingali, S.V.; O'Neill, H.M.; Zhang, Q.; Berglund, L.A. Small Angle Neutron Scattering Shows Nanoscale PMMA Distribution in Transparent Wood Biocomposites. *Nano Lett.* **2021**, *21*, 2883–2890. [\[CrossRef\]](#)
8. Plaza, N.Z.; Jakes, J.E.; Frihart, C.R.; Hunt, C.G.; Yelle, D.J.; Lorenz, L.F.; Heller, W.T.; Pingali, S.V.; Stone, D.S. Small-angle neutron scattering as a new tool to evaluate moisture-induced swelling in the nanostructure of chemically modified wood cell walls. *For. Prod. J.* **2019**, *68*, 349–352.
9. Plaza, N.Z. *Neutron Scattering Studies of Nano-Scale Wood-Water Interactions*; University of Wisconsin-Madison: Madison, WI, USA, 2017.
10. Plaza, N.Z.; Ibach, R.E.; Pingali, S.V. Probing the Nanostructural Mechanisms Behind Decay Resistance of Chemically Modified Wood Using Small Angle Neutron Scattering. In *Proceedings of the 2021 Forest Products Society International Conference, Madison, WI, USA, 15–17 June 2021*; pp. 159–163.
11. Ibach, R.E.; Plaza, N.Z.; Pingali, S.V. Small Angle Neutron Scattering Reveals Wood Nanostructural Features in Decay Resistant Chemically Modified Wood. *Front. For. Glob. Chang.* **2022**, *4*, 1–11. [\[CrossRef\]](#)
12. Plaza, N.Z. On the Experimental Assessment of the Molecular-Scale Interactions between Wood and Water. *Forests* **2019**, *10*, 616. [\[CrossRef\]](#)

13. Plaza, N.Z.; Pingali, S.V.; Qian, S.; Heller, W.T.; Jakes, J.E. Informing the improvement of forest products durability using small angle neutron scattering. *Cellulose* **2016**, *23*, 1593–1607. [[CrossRef](#)]
14. Penttilä, P.A.; Altgen, M.; Carl, N.; van der Linden, P.; Morfin, I.; Österberg, M.; Schweins, R.; Rautkari, L. Moisture-related changes in the nanostructure of woods studied with x-ray and neutron scattering. *Cellulose* **2020**, *27*, 71–87. [[CrossRef](#)]
15. Penttilä, P.A.; Altgen, M.; Awais, M.; Österberg, M.; Rautkari, L.; Schweins, R. Bundling of cellulose microfibrils in native and polyethylene glycol-containing wood cell walls revealed by small-angle neutron scattering. *Sci. Rep.* **2020**, *10*, 20844. [[CrossRef](#)] [[PubMed](#)]
16. Rowell, R.M. Chemical modification of wood: A short review. *Wood Mater. Sci. Eng.* **2006**, *1*, 29–33. [[CrossRef](#)]
17. Ibach, R.E.; Rowell, R.M.; Lee, B.-G. Decay Protection Based on Moisture Exclusion Resulting From Chemical Modification of Wood. In Proceedings of the 5th Pacific Rim Bio-Based Composites Symposium, Canberra, Australia, 10–13 December 2000; Evans, P.D., Ed.; Department of Forestry, The Australian National University: Canberra, Australia, 2000; pp. 197–204.
18. Pingali, S.V.; Urban, V.S.; Heller, W.T.; McGaughey, J.; O'Neill, H.; Foston, M.; Myles, D.A.; Ragauskas, A.; Evans, B.R. Breakdown of Cell Wall Nanostructure in Dilute Acid Pretreated Biomass. *Biomacromolecules* **2010**, *11*, 2329–2335. [[CrossRef](#)] [[PubMed](#)]
19. Nishiyama, Y.; Langan, P.; O'Neill, H.; Pingali, S.V.; Harton, S. Structural coarsening of aspen wood by hydrothermal pretreatment monitored by small- and wide-angle scattering of X-rays and neutrons on oriented specimens. *Cellulose* **2014**, *21*, 1015–1024. [[CrossRef](#)]
20. Rowell, R.M.; Gutzmer, D.I. Chemical Modification of Wood: Reactions of Alkylene Oxides With Southern Yellow Pine. *Wood Sci.* **1975**, *7*, 240–246.
21. Rowell, R.M.; Dale Ellis, W. *Reaction of Epoxides with Wood*; Research Paper FPL 451; Department of Agriculture, Forest Service, Forest Products Laboratory: Madison, WI, USA, 1984.
22. AWWPA E11-97; Standard Method of Determining the Leachability of Wood Preservatives. AWWPA: Hoover, AL, USA, 1999.
23. ASTM D1413; Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures. ASTM International: West Conshohocken, PA, USA, 1999.
24. Goodell, B.; Zhu, Y.; Kim, S.; Kifle, K.; Eastwood, D.; Daniel, G.; Jellison, J.; Yoshida, M.; Groom, L.; Pingali, S.V.; et al. Modification of the nanostructure of lignocellulose cell walls via a non-enzymatic lignocellulose deconstruction system in brown rot wood-decay fungi. *Biotechnol. Biofuels* **2017**, *10*, 179. [[CrossRef](#)]
25. Beaucage, G. Approximations Leading to a Unified Exponential/Power-Law Approach to Small-Angle Scattering. *J. Appl. Crystallogr.* **1995**, *28*, 717–728. [[CrossRef](#)]
26. Beaucage, G. Small-angle scattering from polymeric mass fractals of arbitrary mass-fractal dimension. *J. Appl. Crystallogr.* **1996**, *29*, 134–146. [[CrossRef](#)]
27. Ilavsky, J.; Jemian, P.R. Irena: Tool suite for modeling and analysis of small-angle scattering. *J. Appl. Crystallogr.* **2009**, *42*, 347–353. [[CrossRef](#)]
28. ASTM D2017-05; Standard Method of Accelerated Laboratory Test of Natural Decay Resistance of Woods. ASTM International: West Conshohocken, PA, USA, 2005.
29. Donaldson, L. Cellulose microfibril aggregates and their size variation with cell wall type. *Wood Sci. Technol.* **2007**, *41*, 443–460. [[CrossRef](#)]
30. Lyczakowski, J.J.; Bourdon, M.; Terrett, O.M.; Helariutta, Y.; Wightman, R.; Dupree, P. Structural imaging of native cryo-preserved secondary cell walls reveals presence of macrofibrils composed of cellulose, lignin and xylan. *Biorxiv* **2019**, *10*, 648360.
31. Adobes-Vidal, M.; Frey, M.; Keplinger, T. Atomic force microscopy imaging of delignified secondary cell walls in liquid conditions facilitates interpretation of wood ultrastructure. *J. Struct. Biol.* **2020**, *211*, 107532. [[CrossRef](#)] [[PubMed](#)]
32. Toba, K.; Yamamoto, H.; Yoshida, M. Crystallization of cellulose microfibrils in wood cell wall by repeated dry-and-wet treatment, using X-ray diffraction technique. *Cellulose* **2013**, *20*, 633–643. [[CrossRef](#)]
33. Bhuiyan, M.T.R.; Hirai, N.; Sobue, N. Changes of crystallinity in wood cellulose by heat treatment under dried and moist conditions. *J. Wood Sci.* **2000**, *46*, 431–436. [[CrossRef](#)]
34. Kuribayashi, T.; Ogawa, Y.; Rochas, C.; Matsumoto, Y.; Heux, L.; Nishiyama, Y. Hydrothermal Transformation of Wood Cellulose Crystals into Pseudo-Orthorhombic Structure by Cocrystallization. *ACS Macro Lett.* **2016**, *5*, 730–734. [[CrossRef](#)]
35. Zhu, Y.; Li, W.; Meng, D.; Li, X.; Goodell, B. Non-enzymatic modification of the crystalline structure and chemistry of Masson pine in brown-rot decay. *Carbohydr. Polym.* **2022**, *286*, 119242. [[CrossRef](#)]
36. Zhu, Y.; Plaza, N.; Kojima, Y.; Yoshida, M.; Zhang, J.; Jellison, J.; Pingali, S.V.; O'Neill, H.; Goodell, B. Nanostructural Analysis of Enzymatic and Non-enzymatic Brown Rot Fungal Deconstruction of the Lignocellulose Cell Wall. *Front. Microbiol.* **2020**, *11*, 1389. [[CrossRef](#)]
37. Rowell, R.; Simonson, R.; Hess, S.; Plackett, D.; Cronshaw, D.; Dunningham, E. Acetyl Distribution in Acetylated Whole Wood and Reactivity of Isolated Wood Cell-Wall Components To Acetic Anhydride. *Wood Fiber Sci.* **1994**, *26*, 11–18.
38. Terrett, O.; Lyczakowski, J.; Yu, L.; Dupree, P.; Iuga, D.; Franks, W.; Brown, S.; Dupree, R. Molecular architecture of softwood revealed by solid-state NMR. *Nat. Commun.* **2019**, *10*, 4978. [[CrossRef](#)]
39. Salmén, L. On the organization of hemicelluloses in the wood cell wall. *Cellulose* **2022**, *29*, 1349–1355. [[CrossRef](#)]
40. Thomas, L.H.; Martel, A.; Grillo, I.; Jarvis, M.C. Hemicellulose binding and the spacing of cellulose microfibrils in spruce wood. *Cellulose* **2020**, *27*, 4249–4254. [[CrossRef](#)]

41. Kang, X.; Kirui, A.; Dickwella Widanage, M.C.; Mentink-Vigier, F.; Cosgrove, D.J.; Wang, T. Lignin-polysaccharide interactions in plant secondary cell walls revealed by solid-state NMR. *Nat. Commun.* **2019**, *10*, 347. [[CrossRef](#)] [[PubMed](#)]
42. Felhofer, M.; Bock, P.; Singh, A.; Prats-Mateu, B.; Zirbs, R.; Gierlinger, N. Wood Deformation Leads to Rearrangement of Molecules at the Nanoscale. *Nano Lett.* **2020**, *20*, 2647–2653. [[CrossRef](#)]
43. Arantes, V.; Goodell, B. Current Understanding of Brown-Rot Fungal Biodegradation Mechanisms: A Review. *Deterior. Prot. Sustain. Biomater.* **2014**, *1158*, 3–21.
44. Pingali, S.V.; Urban, V.S.; Heller, W.T.; McGaughey, J.; O'Neill, H.; Foston, M.B.; Li, H.; Wyman, C.E.; Myles, D.A.; Langan, P.; et al. Understanding Multiscale Structural Changes during Dilute Acid Pretreatment of Switchgrass and Poplar. *ACS Sustain. Chem. Eng.* **2017**, *5*, 426–435. [[CrossRef](#)]
45. Petridis, L.; Pingali, S.; Urban, V.; Heller, W.; O'Neill, H.; Foston, M.; Ragauskas, A.; Smith, J. Self-similar multiscale structure of lignin revealed by neutron scattering and molecular dynamics simulation. *Phys. Rev. E* **2011**, *83*, 4–7. [[CrossRef](#)]
46. Yelle, D.J.; Ralph, J.; Lu, F.; Hammel, K.E. Evidence for cleavage of lignin by a brown rot basidiomycete. *Environ. Microbiol.* **2008**, *10*, 1844–1849. [[CrossRef](#)]
47. Yelle, D.J.; Wei, D.; Ralph, J.; Hammel, K.E. Multidimensional NMR analysis reveals truncated lignin structures in wood decayed by the brown rot basidiomycete *Postia placenta*. *Environ. Microbiol.* **2011**, *13*, 1091–1100. [[CrossRef](#)]
48. Hunt, C.G.; Zelinka, S.L.; Frihart, C.R.; Lorenz, L.; Yelle, D.; Gleber, S.C.; Vogt, S.; Jakes, J.E. Acetylation increases relative humidity threshold for ion transport in wood cell walls—A means to understanding decay resistance. *Int. Biodeterior. Biodegrad.* **2018**, *133*, 230–237. [[CrossRef](#)]
49. Jakes, J.E. Mechanism for Diffusion through Secondary Cell Walls in Lignocellulosic Biomass. *J. Phys. Chem. B* **2019**, *123*, 4333–4339. [[CrossRef](#)] [[PubMed](#)]
50. Jakes, J.E.; Hunt, C.G.; Zelinka, S.L.; Ciesielski, P.N.; Plaza, N.Z. Effects of moisture on diffusion in unmodified wood cell walls: A phenomenological polymer science approach. *Forests* **2019**, *10*, 1084. [[CrossRef](#)]
51. Jakes, J.E.; Plaza, N.; Stone, D.S.; Hunt, C.G.; Glass, S.V.; Zelinka, S.L. Mechanism of Transport Through Wood Cell Wall Polymers. *J. For. Prod. Ind.* **2013**, *2*, 10–13.