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NOTE

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An investigation of mercerization in decayed oak wood by a white rot fungus (*Lentinula edodes*)

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Abstract The crystal transformation of cellulose I to cellulose II during alkali swelling was investigated in decayed oak wood that was used for shiitake mushroom cultivation and the results were compared with those of sound wood using X-ray diffraction analysis and ultraviolet microscopy. During mercerization, the sapwood cellulose of decayed wood was easily transformed into Na-cellulose I and then Na-cellulose I was easily converted into cellulose II after washing and drying. The sapwood cellulose of sound wood was converted more slowly to Na-cellulose I and very little Na-cellulose was converted to cellulose II. Na-cellulose I of sound wood can be reconverted to cellulose I during washing and drying. Therefore, it could be concluded that lignin prevented the alkali swelling of wood cellulose and the transformation from cellulose I to cellulose II. The decay of crystalline cellulose might cause an increase in the susceptibility of alkali swelling, so that the degree of mercerization may be also affected.

Key words Cellulose I · Cellulose II · Decayed wood · Mercerization · Na-cellulose I · Ultraviolet microscopy

Introduction

It is known that the solid state irreversible conversion of crystal structure from cellulose I to cellulose II occurs during the mercerization process and Na-cellulose I is an intermediate for cellulose II.[1] Possible mechanisms regarding the conversion of cellulose I to cellulose II were explained by the change of chain polarity[2–4] or chain conformation.[5,6]

Until now, however, the transformation mechanism has not been completely understood. The mercerization of the wood has been studied less than that of nonwoody cellulose samples. Regarding the mercerization of wood cellulose, Revol and Goring[7] suggested that lignin in wood cell walls may prevent the intermingling process during alkali swelling. On the other hand, Murase et al.[8] examined the ultrathin sections of G-layer using the selected area method of electron diffraction, and suggested that lignin does not prevent the alkali swelling of cellulose in wood, but it restrains the intermingling of cellulose chains during alkali swelling and the crystallization of cellulose II. Interestingly, Shiraishi et al.[9] reported that the partial delignification of wood preceding mercerization caused a partial lattice conversion to cellulose II, and the ratio of lattice conversion was increased with the degree of delignification. Lonikar et al.[10] concluded that loosening of the wood texture by an explosion process and trifluoroacetic acid (TFA) treatment, and by introducing a large substituent group[11] was found to have a direct relationship with the lattice conversion of cellulose.

Based upon the experimental results, it is strongly believed that lignin in wood plays a role in the transformation of the cellulose crystal structure during mercerization. Different lignin contents of wood cell walls should therefore affect the degree of mercerization of wood cellulose.

It is suggested that white rot fungi simultaneously degrades lignin along with polysaccharides, or lignin preferentially.[12,13] The concentration of lignin in wood cell walls decreased with the degradation of wood.[14–16] The degraded zone in cell walls may comprise a number of minute pores.[12] The pores could make wood cell walls loosen. *Lentinulla edodes* is a white rot fungus having considerable diversity in delignification capacity among different strains because of a nonselective attack on the cell wall.[13,15]

The objective of this study is to investigate the differences of mercerization between sound wood and decayed wood. The mercerization is discussed with respect to the lignin distribution and crystalline characteristics of the wood cell walls. Lignin distribution in the cell walls of wood samples was examined by ultraviolet (UV) microscopy. Comparison of the degree of mercerization between the sound oak wood and oak wood decayed by a white rot

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fungus was made in an attempt to investigate the crystal transformation during the alkali swelling using X-ray diffraction analysis.

Experiments

X-ray diffraction, mercerization, and delignification

The fine structure of the decayed oak wood that was used for shiitake (*Lentinula edodes* (Berkely) Pegler) cultivation for 5 and 8 years and that of sound wood of *Quercus mongolica* Fischer was examined. Sapwood of the decayed wood was severely degraded. The decayed wood was very brittle and light brown in color. The heartwood of the 5-year decayed wood exhibited almost the same appearance as the sound wood. The color of heartwood was dark brown. The heartwood of the 8-year decayed wood showed some deterioration. The structure of the cellulose crystallites in the specimens were also compared in this study. The degree of crystallinity was calculated with Segal's peak method[17] and the area method.[18] Crystallite widths were estimated from the line profiles of the (200) reflections (d_{200} = 0.39 nm) using the Scherrer equation.[19] The sapwoods of the sound wood and the 5-year decayed wood were treated with 20% NaOH solution until their X-ray diagrams showed complete conversion to Na-cellulose I. Thereafter, the samples were washed with distilled water and dried at room temperature. The alkali-treated wood blocks were delignified with a sodium chlorite solution containing 2 g of $NaClO_2$ and 1.3 g of acetic acid in 100 ml of distilled water at 40°C for 10 days. X-ray diagrams were recorded using a vacuum camera mounted on a Rigaku 2100 V at 40 kV and 40 mA. Na-cellulose samples for X-ray diffraction were put in a glass capillary (diameter = 1.0 mm) to keep samples in a wet condition during the analysis.

Ultraviolet microscopy

Air-dried chips of sapwood were soaked in distilled water and dehydrated with a graded ethanol series, and then embedded in epoxy resin. One-micrometer-thin cross sections were cut from these samples using a diamond knife mounted on a LKB ultramicrotome, and were placed on a quartz slide. Ultraviolet (UV) micrographs were taken at a wavelength of 280 nm with an ultraviolet microspectrometer (Carl Zeiss MPM-03) on Kodak spectrum No.1 film.

Results and discussion

Lignin distribution in cell walls and fine structure

Ultraviolet micrographs of the sapwoods of sound and decayed wood are shown in Fig. 1. The sound wood exhibited a uniformly lignified secondary wall. However, the UV absorption of the decayed wood was low, because the cellulose content was high and the lignin content low.[16] This was especially true in the secondary walls and cell corners of the fibers around parenchyma cells of decayed wood which were severely eroded.

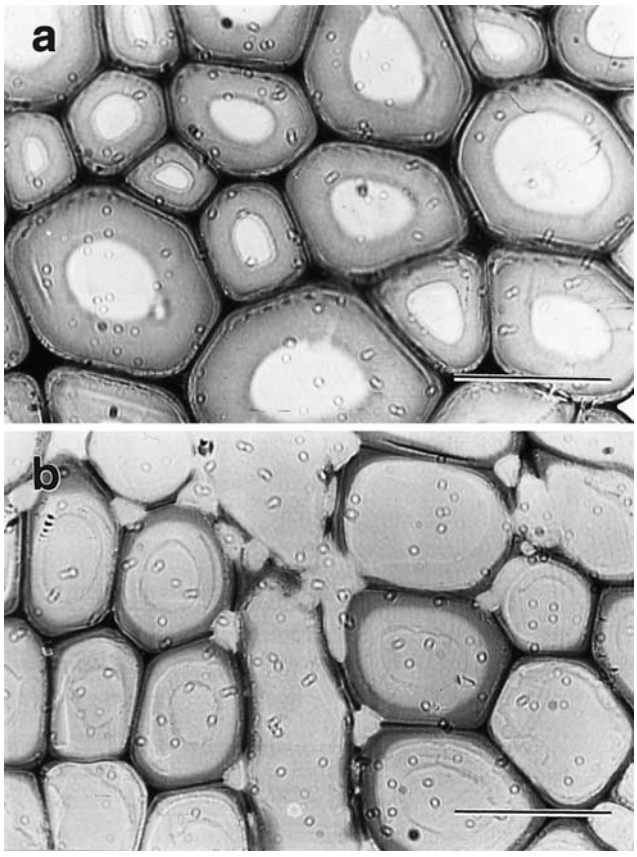

Fig. 1. Ultraviolet micrographs of sound wood (**a**) and 5-year decayed wood (**b**) of *Quercus mongolica. Bars* 30 μm

The X-ray diagrams of the sapwood of sound wood, the 5-year decayed wood, and the 8-year decayed wood, are shown in Fig. 2. The diagrams show cellulose I in all samples, but there were some differences in the fine structure, as given in Table 1. Namely, the crystallinity indices and crystal widths decreased with the duration of decay from about 60% to 40% and from about 3.1 nm to 2.5 nm, respectively. The decrease of crystallinity is in agreement with the results of Cowling's research.[12] He reported that the crystalline material in the decayed wood decreased as the extent of white rot increased.

The change of crystal features in sapwood was greater than that in heartwood. It is well known that the extractives produce resistance to biological fungal attack and that the cell walls in heartwood have more extractives than those in sapwood.[20]

The X-ray diagram of sound wood revealed highly oriented cellulose crystallites. The X-ray diagram of the 5-year decayed wood revealed some orientation; however, the cellulose crystallites of the 8-year decayed wood exhibited a random orientation. It is possible that the change of

Table 1. Fine structures of oak wood samples

Sample	Crystallinity index (%)		Crystal width (nm) (200) reflection
	SM	AM	
5-S	58	50	2.84
5-H	57	51	3.04
8-S	40	34	2.30
8-H	45	39	2.66
S-S	60	52	3.17
H-S	58	50	3.05

SM, Segal's method;[17] AM, area method;[18] 5-S, sapwood decayed for 5 years; 5-H, heartwood decayed for 5 years; 8-S, sapwood decayed for 8 years; 8-H, heartwood decayed for 8 years; S-S, sapwood of sound wood; H-S, heartwood of sound wood

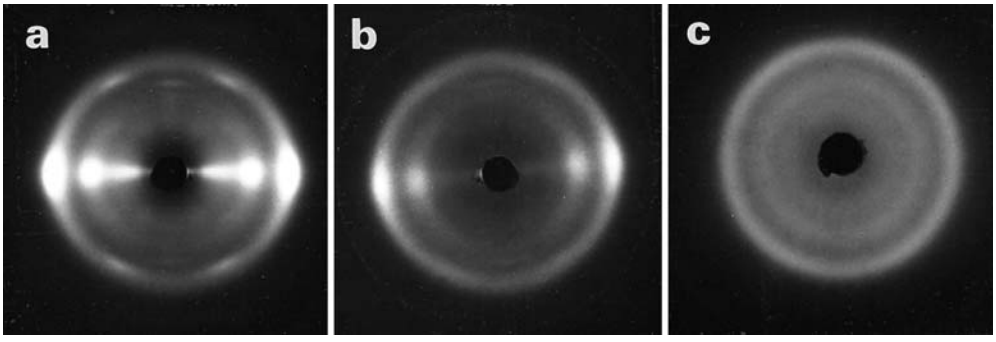

Fig. 2. X-ray diffraction patterns of the sapwoods of sound wood (**a**), and the woods decayed by a white rot fungus for 5 years (**b**) and 8 years (**c**)

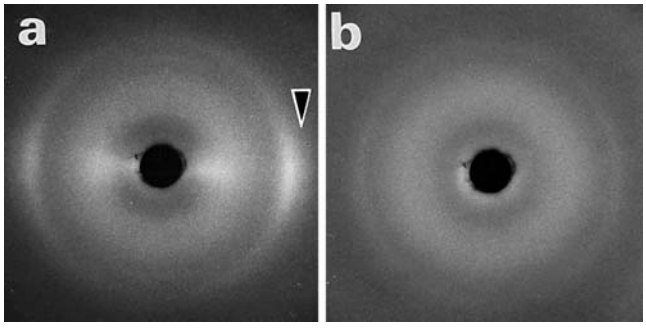

Fig. 3. X-ray diffraction patterns of sound wood (**a**) and 5-year decayed wood (**b**) treated with 20% NaOH solution for 2 hours. *Arrowhead* indicates the (200) reflection (d_{200} = 0.39 nm) in the crystal lattice of cellulose I

cellulose crystallite orientation might have originated from the removal of cellulose microfibrils in the S_2 layer by a cellulase.

Formation of Na-cellulose I

Figure 3 shows the X-ray diagrams of sapwood of sound wood and the 5-year decayed wood treated with 20% NaOH solution for 2 hours. The sapwood of sound wood exhibited a mixture of cellulose I and Na-cellulose I (Fig. 3a), but the sapwood of 5-year decayed wood exhibited only Na-cellulose I (Fig. 3b).

Cellulose I in the sapwood of the decayed wood was more easily transformed into Na-cellulose I in comparison with that of the sound wood. Certainly, the formation of Na-cellulose I in wood was increased as the samples were treated for a longer time, and the formation of Na-cellulose I was faster in the decayed wood than in the sound wood. Cowling[12] demonstrated that the moisture adsorptivity of wood increases with decreasing crystallinity of wood. Therefore, it may be assumed that the decay of crystalline cellulose would affect the formation of Na-cellulose I.

Transformation from cellulose I to cellulose II

The X-ray diagrams of the sound wood treated with 20% NaOH solution for 10 days (Fig. 4a) and the 5-year decayed wood for 1 day (Fig. 4b) showed only Na-cellulose I. Na-cellulose I of sound wood was reconverted into cellulose I by washing and drying. However, Na-cellulose I of the decayed wood showed a mixture of cellulose I and cellulose II. Na-cellulose I of the decayed woods treated for 10 days showed only cellulose II after washing and drying (Fig. 5). It appears that Na-cellulose I of the decayed woods can be easily converted to cellulose II, but cannot be reconverted into cellulose I.

The effect of delignification

Figure 6 shows the X-ray diagrams of the delignified samples after mercerizing for 10 days. The diagrams showed cellulose I in the sound woods (Fig. 6a), and only cellulose II in the decayed wood (Fig. 6b). After the delignification of alkali-treated wood samples, however, the X-ray diagrams

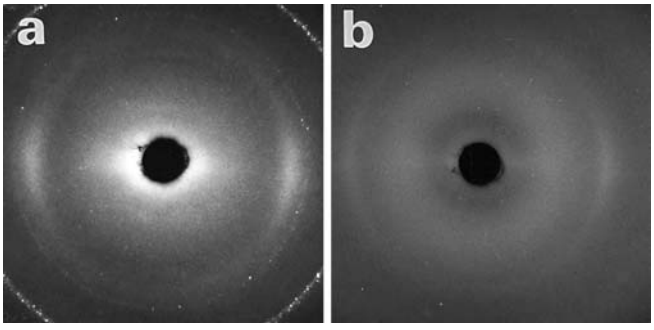

Fig. 4. X-ray diffraction patterns of sound wood (**a**) and 5-year decayed wood (**b**) treated with 20% NaOH solution for 10 days and 1 day, respectively

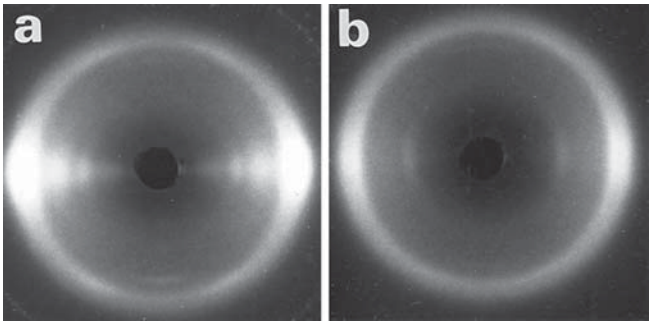

Fig. 5. X-ray diffraction patterns of the 5-year decayed wood treated with 20% NaOH solution for 1 day (**a**) and 10 days (**b**) followed by washing and drying

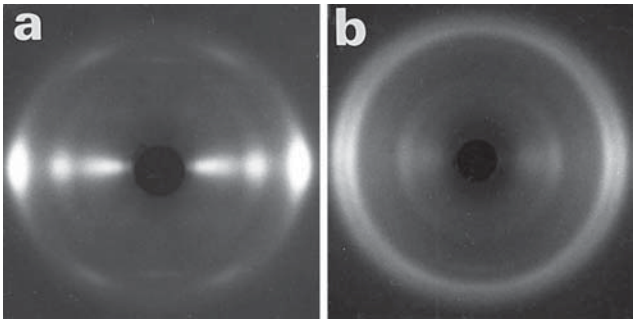

Fig. 6. X-ray diffraction patterns of delignified samples of sound wood (**a**) and 5-year decayed wood (**b**) mercerized for 10 days

showed more clear spots. The clear resolution of the diagrams was probably due to the elimination of background resulting from noncrystalline components of lignin and hemicellulose or the enhancement of crystallization by elimination of matrix substances. From the diagram, it is assumed that the cellulose crystal of alkali-treated wood had the same structure as that of the delignified wood.

These results indicate that the different lignin content and crystalline cellulose in cell walls can change the degree of mercerization of the woods. As proposed in our previous report,[21] cellulose I was transformed into the metastable Na-cellulose I which has an incomplete composition of NaOH and H_2O compared with complete Na-cellulose I ($C_6H_{10}O_5$:NaOH:H_2O = 1:1:3 reported by Sakurada and Okamura[22] and Sobue et al.[23]). That is, when wood cellulose is swollen by alkali solution, lignin in the cell walls packs around the microfibrils and prevents the penetration of Na^+ ion into the cellulose chains, resulting in the restriction of the formation of complete Na-cellulose I and the swelling of cellulose chains. Thereafter, the metastable Na-cellulose I can be regenerated to cellulose I by washing and drying. Otherwise, the stable Na-cellulose I formed in wood during alkali swelling could be transformed into cellulose II after delignification.

Decayed woods exhibited not only low lignin concentration and poor crystalline characteristics, but also high porosity in cell walls. The low lignin concentration and decay of crystalline cellulose might cause an increase in the susceptibility of alkali swelling, which may also affect the degree of mercerization. It is thought that the cellulose of decayed wood cell walls can be easily transformed into stable Na-cellulose I, and then stable Na-cellulose I into cellulose II by washing and drying.

Conclusions

Cellulose I of the sapwood of decayed wood that has a low lignin concentration can be easily transformed into Na-cellulose I and subsequently from Na-cellulose I into cellulose II during mercerization. However, cellulose I of the sapwoods of the sound wood that has a high lignin concentration is converted more slowly to Na-cellulose I, and very little Na-cellulose I is converted to cellulose II. Na-cellulose I of sound wood can be reconverted to cellulose I during washing and drying. Thus, it can be concluded that lignin prevents the alkali swelling of wood cellulose and consequently the transformation from cellulose I to cellulose II. The degradation of crystalline cellulose might cause an increase in the susceptibility of alkali swelling, so that the degree of mercerization may be also affected.

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