Using Lignin Content, Cellulose Content, and Cellulose Crystallinity as Indicators of Wood Decay in *Juglans mandshurica* Maxim. and *Pinus koraiensis*

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The main chemical indicators for healthy wood and rotted wood at different decay levels in two species, namely *Juglans mandshurica* Maxim. and *Pinus koraiensis,* were preliminary analyzed. The cellulose content, lignin content, and relative crystallinity were measured using the nitric acid-ethanol method, acid-insoluble lignin, and X-ray diffraction to further explore the process of wood decay. Results indicated that the cellulose content and relative crystallinity decreased and the acid-insoluble lignin content increased as wood decay increased. X-ray diffraction results showed that there were no significant changes in the lattice structure between healthy wood and rotted wood. Approximately 98.3% and 99.9% of the variations in wood decay for *Juglans mandshurica* Maxim. and *Pinus koraiensis*, respectively, can be explained by the comprehensive effect of the above chemical indicators.

Keywords: Wood decay; Cellulose content; Acid insoluble lignin; Relative crystallinity

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INTRODUCTION

As a kind of natural organic material, wood is widely preferred for building applications because it is cost-effective, low in processing energy, easy to work with, renewable, strong, and aesthetically pleasant (Usta 2006). Growth of the wood products industry has been accompanied by a significant expansion in the use of wood in outdoor applications (Huang *et al.* 2012). However, ultraviolet radiation, fire, worms, and fungi can cause wood deterioration in connection with chemical changes, physical changes, and structural changes. Among these factors, the fungi are known to be responsible for initiating the degradation of wood (Worrall 2011), and it is one of the most common wood defects. Wood decay can have a significant impact on wood quality and grade.

In China, the occurrence of wood decay in over mature forests in the main forest districts has been shown to be above 40 percent (Li 2002; Chi 2003). Therefore, wood decay can greatly affect the production of forest products and wood utilization. Wood decay is caused by various kinds of wood fungi, of which the three main types are recognized as white-rot, brown-rot, and soft-rot (Pandey and Pitman 2003). Brown-rot fungi selectively consumes structural carbohydrates, and white-rot fungi consumes all structures (Wang *et al.* 2000; Li 2002). The crystallinity of decayed wood is changed, which can lead to the degradation of cellulose, hemicellulose, and lignin. The cellulose, hemicellulose, and lignin that wood cells are made of are the necessary nutrition sources for fungi. Previously, evaluation of the extent of decay by a single fungi, either brown-rot

fungi or white-rot fungi, was performed by noting physical characteristics, using microscopic observations (Grinda 1997; Ali *et al.* 2011), spectral analyses (Pandey and Pitman 2003; Xu *et al.* 2013), or conventional chemical analyses (Gelbrich *et al.* 2008; Gelbrich 2009; Liu *et al.* 2009). However, wood is rotted by several kinds of fungi, rather than a single type of fungus, in the field. The environmental change has a direct impact on the growth of wood decay fungi and further affects the relative degradation speed of all chemical components. It is therefore necessary to analyze the chemical composition of decayed wood and to investigate the relationships among chemical composition, crystallinity, and the state of degradation.

In this paper, we preliminarily analyzed the variation in the chemical index for healthy wood and rotted wood at different decay levels in two species, namely *Juglans mandshurica* Maxim. and *Pinus koraiensis*, from Xiaoxing'an Mountain of China. The results will provide theoretical references for the determination of wood decay and decay control.

EXPERIMENTAL

MATERIALS

Samples and treatment

A total of 16 samples including healthy logs and rotted logs from two species, *Juglans mandshurica* Maxim. and *Pinus koraiensis*, were obtained from the Dongfanghong Forest Farm of the Fangzheng Forestry Bureau in Heilongjiang province, China, in April 2012. Then, the sample logs were stored in a log yard for six months to reach the air-dried condition. The water content of the sample logs was measured. A 10-cm-thick disc was cut 1.2 m above away from the root. Both healthy wood and rotted wood were collected from the same species. The degree of wood decay was determined using the percentage of the area of decay parts to the area of disc (Fig. 1). After being ground to powder and air dried, the samples were stored in ground-glass stoppered flasks; the samples that passed through a 40- to 60-mesh sieve were used for chemical analysis, and those that passed through a 60-mesh sieve were used for crystallinity analysis.

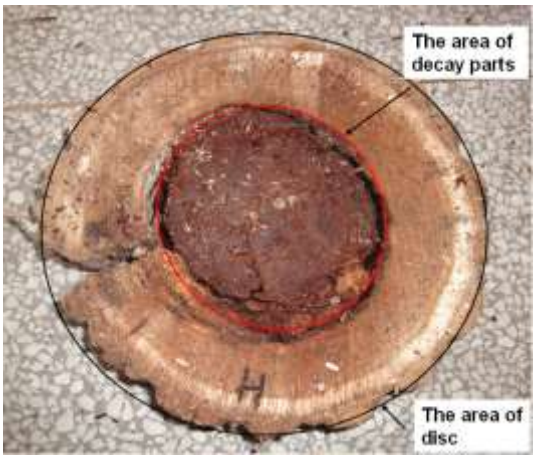

Fig. 1. The percentage of the area of decay parts to the area of disc

Reactant and equipment

In this study, a 10% barium chloride solution was used. The analytical reagents included nitric acid, ethanol, benzol, and sulfuric acid. To determine crystallinity, X-ray diffraction was carried out with a D/MAX-3B (Rigaku Inc., Japan) using a copper target X-ray tube and a nickel sheet liner to eliminate Kα radiation. Other settings included a voltage of 40 kV and an electric current of 30 mA, with a $\theta/2\theta$ linked scanning method interval of 0.02° and a preset time of 2 s. The monochromator was curved-crystal graphite, and the slits set as follows: divergence slit *Ds* = 1°, anti-scatter slit *Ss* = 1°, and receiving slit *Rs* = 0.3 mm. The detection device was a flicker counter.

METHODS

Measurement of wood chemical composition and relative crystallinity

The cellulose content was measured using the nitric acid-ethanol method. The content of acid-insoluble lignin was measured based on the Chinese national standard GB/T 2677.8 (1994). The relative crystallinity was measured using X-ray diffraction. The sample powder was placed on the sample stage. The 2θ intensity curve was measured using the $\theta/2\theta$ linked scanning method with a scanned area of 10°~40° (2θ). Each sample was measured twice, and the average value was used as the measurement result. The maximum and minimum values can be identified at 2θ = 22° and 2θ = 18°, respectively, in the scanned curve. The relative crystallinity of the sample was determined using the Segal method based on the two values (Segal *et al.* 1959).

RESULTS AND ANLYSIS

Variation in Chemical Composition

The variations of cellulose content and acid-insoluble lignin in the healthy and rotted wood by species are illustrated in Figs. 2 and 3. The cellulose content decreased by 44.12% for *Juglans mandshurica* Maxim. and by 23.44% for *Pinus koraiensis* as the degree of wood decay increased to 40%. The acid insoluble lignin increased by 56.92% and 26.43% for the two species, respectively. The results were consistent with those obtained by Li (2009) and Liu (2006). The variation of chemical composition during wood decay can be explained from two perspectives: chemical degradation and bio-degradation.

First, cellulose is an organic compound with the formula $(C_6H_{10}O_5)n$. It is a polysaccharide that consists of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units (Li 2002). Because of its structural characteristics, cellulose can have several chemical reactions under certain conditions, including the degradation of cellulose chains and cellulose hydroxylation. These reactions are affected by hydrolysis reactions, alkaline degradation, oxidative degradation, microbial degradation, and mechanical degradation. Lignin is a complex polymer, the chief non-carbohydrate constituent of wood, which binds to cellulose fibers and hardens and strengthens the cell walls of plants (Li 2002). Lignin degradation processes include acid degradation, hydrolysis, mild hydrolytic degradation, and oxidative degradation. Second, the decomposition of wood fungi will produce all kinds of cellulosic enzymes and lignin enzymes that can be used to decompose cellulose and lignin. Under acidic conditions,

cellulose will generate amorphous cellulose and soluble oligose by reacting with endo-glucanase and then become degraded to glucose under a direct reaction with exo-glucanase; it may also generate cellobiose and then be degraded to glucose under the function of the cellobiose enzyme. The generated glucose will be partially used to synthesize cellular material and partially oxidize carbon dioxide and water (Kirk and Farrell 1987; Higuchi 1990). Our research indicated that among all the microorganisms that can degrade lignin, only white-rot fungi can completely decompose lignin and degrade the complex lignin macromolecule into carbon dioxide and water, while others can partially change the molecule structure of lignin instead of providing thorough decomposition.

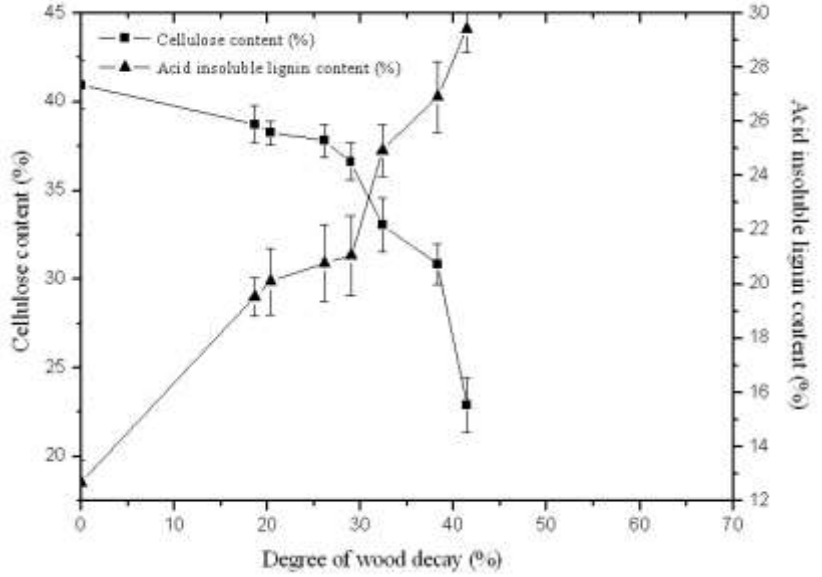

Fig. 2. Changes in chemical composition of *Juglans mandshurica* Maxim.

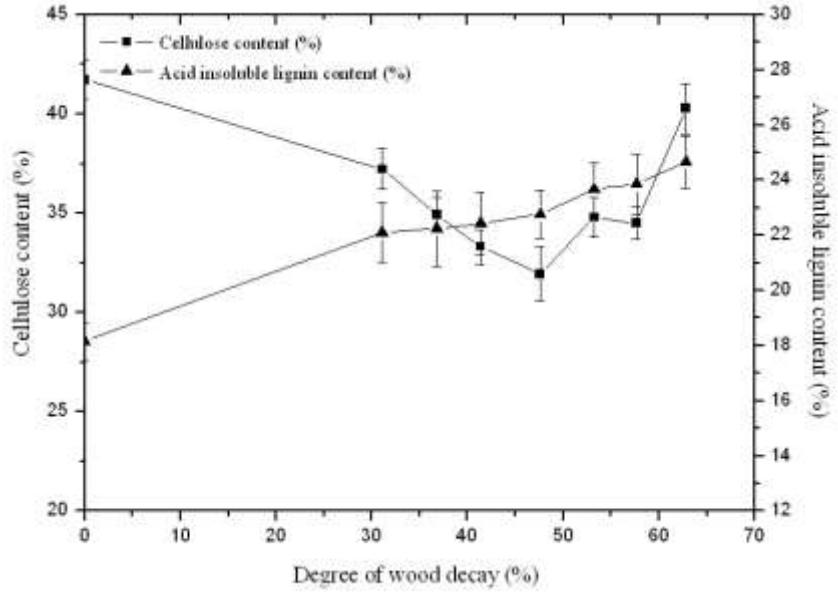

Fig. 3. Changes in chemical composition of *Pinus koraiensis*

Wood fungi include white-rot fungi and brown-rot fungi. The enzymes generated from the two types of wood fungi have different abilities to decompose cellulose and lignin. White-rot fungi can completely decompose lignin; however, the degrading effect on cellulose is not appreciable. That is to say, if wood encounters white-rot fungi, the cellulose content will not decrease. Conversely, brown-rot fungi can easily decompose cellulose and obtain nutrition for growth from the process. Figure 3 shows that the cellulose content of *Pinus koraiensis* increased as wood decay increased from 48% to 63%. This is because the samples interacted primarily with white-rot fungi, which are very common for *Pinus koraiensis* in this stage.

Variation in Relative Crystallinity

The crystallinity of wood cellulose is an important descriptive parameter for the cellulose super-molecular structure. The relative crystallinity of healthy wood and decayed wood by species is illustrated in Fig. 4. The crystallinity differs at different sample locations (heartwood and sapwood) in the same wood. The crystallinity for heartwood will be higher than that for sapwood. In this study, the degree of wood decay was the only factor considered during sampling, and the difference in the crystallinity at different sample locations was ignored. Without considering the influence of the sample locations (heartwood and sapwood), the relative crystallinity of the decayed wood decreased compared to the healthy wood. The results were consistent with the findings from Liu *et al*. (1989) and Andersson *et al.* (2003) on the crystallinity measurement of Korean Pine (*Pinus koraiensis*), birch (Betulaceae), and Amur cork tree. The oxidative degradation during the decay process leads to the breakdown of giant cellulose molecules; alternatively, the oxygen bridge that connects the ground ring of the giant molecule may collapse, destroying partial cellulose crystal regions. Either of these actions will result in a change from a directional ordered arrangement to a random arrangement and a transition from crystalline regions to non-crystalline regions.

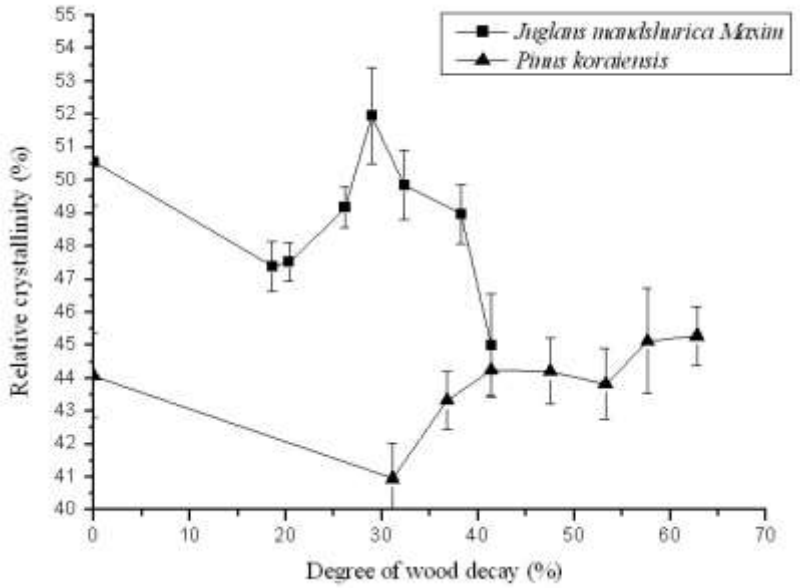

Fig. 4. Changes in crystallinity in *Juglans mandshurica* Maxim. and *Pinus koraiensis*

Figure 5 shows the X-ray diffraction diagrams by species. The crystallinity of the healthy wood and the decayed wood were compared by one-way ANOVA analysis and LSD test at the significance level of 5%. There were no appreciable changes in the X-ray diffraction between the healthy wood and the decayed wood ($p>0.05$). The peak value occurred at 2θ = 22°, and the minimum occurred at 2θ = 18°, for the two species. Therefore, wood decay only altered the degree of crystallinity, but not the lattice structure of cellulose.

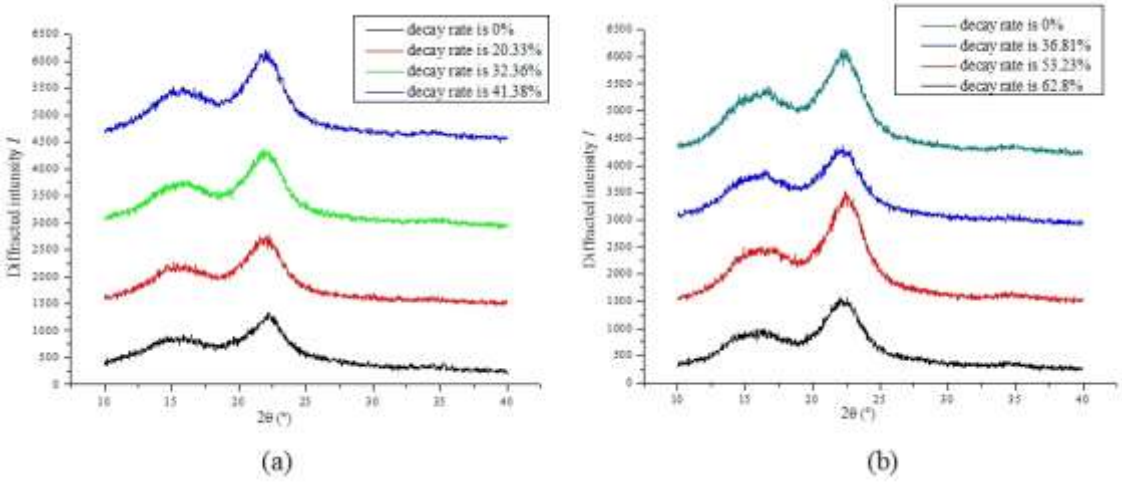

Fig. 5. X-ray diffraction diagrams of (a) *Juglans mandshurica* Maxim. and (b) *Pinus koraiensis*

Response of Chemical Factors to Wood Decay

Cellulose and lignin are the important nutrition sources during the growth of wood fungi. Water content should also be considered during this process. In addition, wood decay may cause a change in the crystallinity. The response of wood decay to the comprehensive effect of these factors was analyzed. Single-factor ANOVA was conducted for wood decay and each of the four factors (Table 1). Multi-regression models were built for the two test species, *Juglans mandshurica* Maxim. and *Pinus koraiensis*, based on the experimental data (Table 2). The results indicated that the degree of wood decay was significantly correlated with cellulose content and acid-insoluble lignin content for *Juglans mandshurica* Maxim. (Table 1). There is also a significant correlation between the degree of wood decay and acid-insoluble lignin content for *Pinus koraiensis*. The variation of wood decay can be better explained by the comprehensive effect of cellulose content, acid-insoluble lignin, water content, and crystallinity, with the coefficient of determination 0.983 for *Juglans mandshurica* Maxim. and 0.999 for *Pinus koraiensis*, respectively (Table 2).

Table 1. Correlation Coefficients between Degree of Wood Decay and Primary Chemical Composition

Species	Cellulose content	Lignin content	Water content (%)	Crystallinity
Juglans mandshurica Maxim.	-0.834*	0.975**	0.105	-0.370
Pinus koraiensis	-0.459	0.987**	0.232	0.408

*$P < 0.05$, significant correlation; **$P < 0.01$, extremely correlated

Table 2. Multiple Regression Model of the Degree of Wood Decay

Species	Regression model	R^2	P value
Juglans mandshurica Maxim.	Y=134.949+0.379x_1+3.123x_2+1.306x_3+1.489x_4	0.983	0.034
Pinus koraiensis	Y=-227.657-0.394x_1+9.314x_2+1.998x_3+1.286x_4	0.999	0.000

Y-degree of wood decay (%), x_1-cellulose content (%), x_2-acid insoluble lignin content (%), x_3-water content after air drying (%), x_4-relative crystallinity (%), R^2-coefficient of determination, P-significance ($P<0.05$ indicates significant correlation, while $P<0.01$ indicates extremely significant correlation)

PROPOSED FUTURE WORK

This paper has described preliminary work for analysis of the variation in the chemical index for healthy wood and rotted wood at different decay levels. It would be worth carrying out careful follow-up work in which care is taken to keep track of such factors as juvenile and mature wood, sapwood, and heartwood. In another kind of follow-up work, matching samples of wood (maybe discs or lumber) could be subjected to different types of rot fungal attack for different periods or under different conditions, making it possible to rule out effects attributable to factors such as variable chemical content within a species, early *vs*. mature, and heart *vs*. sapwood.

CONCLUSIONS

1. Wood decay was found to affect the cellulose and acid-insoluble lignin content. The cellulose content and relative crystallinity decreased and the acid-insoluble lignin content increased as wood decay increased.
2. The relative crystallinity decreased due to wood decay; however, there is no change in the lattice structure. There was still a clear X-ray diffraction pattern in the rotted wood.

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